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Fatty Liver-Hemorrhagic Syndrome (FLHS) in Caged Laying Hens

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FATTY LIVER-HEMORRHAGIC SYNDROME (FLHS) IN CAGED LAYING HENS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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RAN

FATTY LIVER-HEMORRHAGIC SYNDROME (FLHS) IN CAGED LAYING HENS

Abstract

RICHARD A. NELSON

Under the supervision of Professor C. W. Carlson

A series of eleven experiments was conducted with caged laying hens to study the effects of diet and strain on production and fat accumulation in the liver. In Experiments 1 to 4, a normal ad libitum feeding regime was used, while in Experiments 5 to 11, a force-feeding technique was used as a procedure to increase the incidence of FLHS. Four commercial hybrid strains and a straightbred SCWL (Regional Control) strain were utilized in this study.

Choline supplementation at 1500 mg/kg of diet increased egg production from 1 to 11% while decreasing total liver lipid as much as 50%. Supplementation of biotin had little beneficial effect in preventing FLHS.

The level of dietary protein affected egg production but had no effect on liver fat accumulation. When supplementing excess methionine in a low protein diet (14%) to increase the availability of methyl groups, a significant increase in total liver lipid resulted (11 g). Increasing the level of added dietary fat resulted in no significant increases in liver lipids but rather produced a tendency for decreased liver size and lipid content.

Corn, oats and wheat were used as primary carbohydrate sources to test for effects on FLHS. Conflicting results from feeding wheat as

the only grain source resulted between two experiments. A highly significant decrease in total liver lipid occurred in a force-feeding regime, while no effect was observed between corn and wheat diets in an ad libitum experiment. Egg production averaged 10% less for hens fed oats as the only grain source as compared to those on a corn diet. However, a 50 to 75% decrease in total liver lipids was also observed, suggesting that a factor in oats is necessary for normal lipid metabolism in laying hens. Results from two experiments using oat hulls and wheat bran indicate that these parts of oats and wheat, respectively, are not the factor responsible for the liver lipid lowering effect.

The results of excessive energy intake by force-feeding suggest that total caloric consumption plays an important role in the incidence of FLHS. Force-feeding at levels from 114 to 162% of ad libitum fed hens resulted in three- to fivefold increases in total liver lipids and hemorrhage score. Large differences in liver lipids between strains suggest there is a difference in genetic susceptibility to FLHS.

Histological observations verified that hens in normal egg production generally have high levels of liver lipid. Photomicrographs and electron micrographs suggest, however, that excessive liver lipid will lead to the presence of extracellular lipid and disarrangement of the hepatic nuclei and mitochondria, resulting in FLHS.

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INTRODUCTION

More than two decades have passed since Couch (1956) first described a condition of excess fat accumulation in the livers of laying hens that were submitted to the Texas Diagnostic Laboratory. He named the condition fatty liver syndrome (FLS) and stated it to be generally characterized by:

"A. An increase in body weight of 25 to 30%.
B. A decrease in the rate of egg production by up to 30%.
C. An apparently healthy bird in good condition.
D. An excess of abdominal fat, fatty livers, liver capillary hemorrhages and liver hematomas.
E. An increase in mortality of the laying hens, particularly on warm days."

Couch reported that the fat content of the livers varied greatly but often reached levels as high as 70% (dry matter basis) and that globules of fat seeped through the liver capsule.

Since this first report, many subsequent reports have appeared in the literature describing the syndrome in more detail. Reedy (1968) characterized the disease similarly but reported decreases in egg production as high as 50% and a liver which was greatly increased in size, very fatty, extremely friable and varying in color from tan to pale yellow. Hafez (1955) reported that normal liver size in laying hens usually runs between 2 and 3% of the body weight. Since liver weights of birds with this condition often exceed 4%, these higher values would have to be considered abnormal. Other researchers such as Shivaprasad and Jaap (1977) or Nesheim et al. (1969) argue that large, moderately fat livers are needed in high producing hens. They

stated that the rate of yolk synthesis appeared to be dependent on liver weight and total liver lipid. A hen, producing an egg per day, excretes through the egg yolk about 6 g of lipid per day. Therefore, the liver must be very active in lipid synthesis to maintain this production.

Harms et al. (1972) reported that serum lipid levels in birds that had quit laying due to FLS were three times as great as in other laying hens in the same flock. The latter two reports therefore suggest two reasons for liver fat being high in laying hens with FLS, (1) the liver loses its ability to transport lipids efficiently or (2) the liver lipid level is required to be critically high in order to maintain high egg production.

Wolford and Polin (1972c) suggested a change in the name of the liver condition to "Fatty Liver-Hemorrhagic Syndrome (FLHS)," since it better characterizes birds that die from excessive liver lipid. Therefore, from this point on, the syndrome will be referred to as FLHS.

To date, research reports on the effect of diet or several dietary additives on liver lipids are quite conflicting. Therefore, 11 experiments were conducted using several strains of birds to test some dietary factors that may have an effect on liver lipid accumulation in the caged laying hen. A force-feeding technique similar to the one devised by Wolford and Polin (1972b) was used in seven of the experiments to increase the incidence of the disease.

Also reviewed in this paper will be a similar condition in young broilers that is referred to as "Fatty Liver and Kidney Syndrome (FLKS)." The etiology of this disease and its similarity to FLHS will be discussed.

REVIEW OF LITERATURE

Investigations have been conducted and reported in the literature on many factors affecting lipid metabolism as it relates to its accumulation in the livers of laying hens and broiler chicks. Some of these factors include several lipotropic agents, other vitamins, protein or amino acid levels, energy source or level, minerals, type of environment, age of the birds and toxic substances. This review of literature will contain reports of each of these general topics.

Effect of Lipotropic Agents and Other Vitamins on FLHS

Lipotropic agents have most often been suspected of being deficient in diets of flocks with a high incidence of FLHS. Choline deficiency is often implicated as a cause of fatty liver because in the rat its supplementation will overcome fatty livers that are produced from low protein, low methionine and high fat diets. In this instance, the problem is primarily a methyl group deficiency since, if methionine is supplied, choline is not needed.

Choline deficiency can readily be produced in young chicks by leaving it out of a purified diet. Methionine, however, does not correct such a drastic deficiency. Fatty liver is not observed but instead the birds show perosis and grow slowly. After 8 weeks of age, the chicken at times appears to have the ability to synthesize adequate choline as far as growth and/or later egg production is concerned (Nesheim *et al.*, 1969, 1971; Lucas *et al.*, 1946; Ringrose and Davis, 1946; Bossard and Combs, 1970; Johnson, 1954; Skinner *et al.*, 1951;

Crawford et al., 1967; Balloun, 1956; Chah et al., 1975; Gish et al., 1949). However, others have shown that choline supplementation will at times increase egg production (Welch and Couch, 1955; Soloma et al., 1965; Holmes and Kramer, 1965; Schexnailder and Griffith, 1973; Griffith and Schexnailder, 1972; Griffith et al., 1969).

Effectiveness of choline supplementation in the prevention of fat accumulation in the liver is also controversial. Although Jensen et al. (1974b), Crawford et al. (1969), Schexnailder and Griffith (1973), Abbott and DeMasters (1940), Griffith and Schexnailder (1972) and Griffith et al. (1969) showed a significant effect of choline on decreasing the liver fat, other researchers (Hamilton and Garlich, 1972; Bossard and Combs, 1970; Nesheim et al., 1969, 1971; Chah et al., 1975; Wolford and Murphy, 1972) were unable to show with practical diets any effect of choline on liver fat. Nesheim et al. (1971), however, using purified diets observed some decrease in liver fat due to choline.

Crawford et al. (1969) tried to determine the requirement for choline by feeding several levels. It was concluded that 600 mg/kg of diet are sufficient to maintain maximum egg production, while liver lipid was significantly decreased ($P < .05$) by an additional 3 to 4% (dry matter basis) when 5700 mg/kg were fed. Work conducted by Griffith et al. (1969) and Schexnailder and Griffith (1973) showed that choline supplementation decreased liver fat by 10 to 12% with additional decreases obtained by further supplementation with methionine and vitamin B_{12}. Jensen et al. (1974b) showed that a

lipotropic mix of choline, B_{12}, vitamin E and inositol decreased liver lipid by 16% or by about one-half based on total liver lipid.

A mixture of choline, vitamin B_{12}, inositol and vitamin E did not prevent hemorrhages characteristic of FLHS in Wolford and Murphy's work (1972). Liver lipid *per se* was not related to hemorrhages. In fact, 51.3% of the birds receiving a high energy diet (2.9 kcal/g) had lipid values equal to or greater than the 12.7% of the birds having hemorrhages. Thus, total liver lipid is not the only factor involved. They suggested increased liver size as a possible mechanism by which hemorrhages occur. Possibly as the liver increases in size, tensions on the vascular system may aid in rupturing of vessels.

Nesheim *et al.* (1969) concluded from 3 years of experimental additions of .2% choline that mortality from liver hemorrhage generally occurs in birds with very high fat content (average, 65%, dry matter basis). This mortality peaked in April through June when the weather was warming up. High levels of liver fat *per se* did not seem to interfere with production or health of the bird. Since a dietary deficiency did not produce liver lipid accumulation in their work, they suggested that biosynthesis had occurred. Prevention of choline synthesis can be influenced by factors not readily understood which may result in a need for dietary choline under some circumstances. They also warned that drawing conclusions from treatments in field cases may not always be easy, since spontaneous decline in mortality occurs many times.

Vitamin B_{12} deficiency is often implicated in fatty livers because it is involved in methyl group transfer, which in turn affects the biosynthesis of choline (Nesheim et al., 1969; Welch and Couch, 1955). Therefore, when vitamin B_{12} is added to choline deficient diets, improvements in egg production have been noted (Welch and Couch, 1955; Holmes and Kramer, 1965; Griffith et al., 1969; Schexnailder and Griffith, 1973). Small decreases in liver lipid were noted with the additions of vitamin B_{12} (Griffith et al., 1969; Schexnailder and Griffith, 1973; Jensen et al., 1974b), but decreases were not significant unless choline supplementations were also made.

Reedy (1968) proposed that a mixture of choline, inositol, B_{12} and vitamin E would reduce the incidence of FLHS. These findings were supported by Jensen et al. (1974b). However, several other investigators have reported no significant effect of adding one or all of the vitamins in this mixture. Feeding from 750 to 5000 mg of inositol per kg of diet (Bossard and Combs, 1970; Pearce, 1972; Leveille and Bray, 1970; Ragland et al., 1970; Griffith and Schexnailder, 1972; Wolford and Polin, 1975) resulted in no significant effects on measured performance criteria including egg production, egg size, body weight, serum lipids or percent liver fat. Feeding the total vitamin mixture in a diet containing 10 µg of aflatoxin per g to test its ability in preventing FLHS (Hamilton and Garlich, 1972) clearly showed that the added vitamins had no effect on development of FLHS as caused by aflatoxin or on the recovery when aflatoxin was removed from the diet. They had previously shown (Hamilton and Garlich, 1971) that feeding aflatoxin

can experimentally produce FLHS in about 2 weeks. In this case, feeding 0, 1.25, 2.5, 5.0, 10.0 or 20.0 ug aflatoxin per g of feed resulted in liver lipid values of 36, 33, 43, 59, 55 and 55% (dry basis), respectively. Liver weight, as a percent of body weight, increased by an average of 1%, while pancreas or spleen weights were not affected. Wolford and Murphy (1972) also found a vitamin mixture of choline, inositol, B_{12} and vitamin E to be ineffective in preventing FLHS.

Since selenium is involved as a co-factor for pancreatic lipase in the body, several researchers have tested its effect on preventing FLHS. Jensen et al. (1974a) supplemented a corn-soybean meal diet with 1 ppm selenium in two experiments. In the first experiment, this addition significantly reduced liver lipid between 6 and 8% or about 5 g of total liver fat. In the second experiment, selenium added to the diet resulted in hens with liver lipids of one-half that of hens on the basal diet (7 vs. 14 g per liver). However, variability among hens was such that the differences did not prove to be statistically significant. In contrast, work by Wolford and Murphy (1972) and Griffith and Schexnailder (1972) clearly showed that selenium additions had no effect on liver lipid accumulation.

Several other B vitamins have been tested for their possible role in FLHS. Folic acid, which is involved in methyl group transfer, can affect choline biosynthesis. However, Schexnailder and Griffith (1973) found no effects on any parameters measured when it was added to the diet. In addition, the supplementation of riboflavin, pyridoxine,

calcium pantothenate or biotin had no beneficial effects in their work. Chah et al. (1975) showed no effects on egg production, liver fat or incidence of hemorrhage when feeding 1.1 mg/kg of biotin. In addition, the feeding of supplemental niacin and/or biotin to 60-week-old hens (Jensen et al., 1976b) resulted in no reduction in liver fat. The authors stated, however, that the birds may have had sufficient storage of vitamins for the duration of the 12-week experiment.

Effect of Source and Level of Fat on FLHS

Adding fat to the ration of laying hens had produced conflicting effects on production and FLHS. Price et al. in 1957 reported that hens in cages developed a fatty liver condition as a result of or prior to a drop in egg production. Feeding 0 to 14% poultry oil had little effect on liver percent fat as long as the ratio of calories to protein was between 100 and 125 (kcal/kg:1% protein). Later work from this same station (McDaniel et al., 1959) showed that the presence of up to 10% animal or vegetable fat had no effect on liver parameters. Excessive deposition of abdominal fat was observed in approximately the same degree and incidence as FLHS. This abdominal fat appeared to be closely related to an increase in body weight. However, poultry oil or soybean oil had little effect on the amount of abdominal fat present. Later work by Chah et al. (1975) was in close agreement with this report. They showed that 10% additions of corn or yellow grease resulted in an improvement of feed efficiency and egg size and a small increase in final body weight. However, the control livers showed

somewhat more fat by visual score and actual fat analysis than those from the added fat diets.

Jensen et al. (1974b) and Ragland et al. (1970) also showed no change in liver parameters measured from adding 0 to 7% animal fat. Feeding 5 to 10% animal fats to birds on litter (Weiss and Fisher, 1957), however, showed changes in lipid metabolism during the laying cycle. They showed that the fat additions caused elevated plasma cholesterol and total plasma lipid, excess deposits of body fat, friable and fatty livers, fatty deposits in and around the kidneys and a greater severity of aortic atherosclerosis. Bragg et al. (1973) found that the source of fat may account for some of these conflicting reports. When substituting 0, 1, 2, 4 and 8% tallow, soybean, sunflower or rapeseed oil isocalorically for glucose in a wheat-soybean meal ration, the results indicated that the nature of dietary fatty acid composition is more important than the amount of energy. Fatty livers ranging from 40 to 50% (dry matter basis) were observed with the 8% animal tallow and rapeseed oil diets, whereas soybean and sunflower oil showed protection against fat accumulation at all levels, with the livers showing only 20 to 25% fat.

Effect of Energy Level and Force-Feeding on FLHS

The age-old European tradition of producing "pate de foie gras," by cramming geese with excessive grain for 3 to 4 weeks until necrotic damage to the developing fatty liver occurs, was first tried with laying hens by Wolford and Polin (1972a) to see if FLHS could be

produced at will. This technique was described in detail (Wolford and Polin, 1972b) and recommended as a way of producing livers that are typical of FLHS in commercial flocks. Several experiments employing the force-feeding technique have since been reported indicating excessive energy intake as a probable cause of FLHS (Ivy and Nesheim, 1973; Wolford and Polin, 1974, 1975).

Restricted feeding and force-feeding at 50 to 150% of the daily feed intake of control hens was used in three experiments that lasted for 21 days each (Wolford and Polin, 1974). Restricted feeding for two 1 1/2 hour periods each day resulted in 73% intake of control value. This resulted in body weight loss without affecting production during the 21-day experiment. Increased force-feeding at intervals of 50, 100, 112.5, 125 and 150% of the control birds' consumption reduced ad libitum eating from 47 g down to 4 g per bird per day. Body weight gain during the experiment was directly proportional to the total feed intake of the hens. Egg production decreased in the 125 and 150% force-feeding regimes. Feed consumption was 63 g per day for the restricted birds and 87 g for the control birds. Feed intake increased to 145 g per day for the birds force-fed at 150% of normal consumption. This increased feed intake resulted in the liver weight increasing from 24 g in the control to 107 g in the 150% group. Total liver lipid increased from 1.6 to 44.1 g, respectively. Close examination of individual bird data for the livers indicated that, on a relative basis, water and nonlipid dry matter increased at an equivalent rate as the daily feed intake exceeded the control intake. However, the

hepatic lipid content increased as much as 23-fold above the respective control value for the upper force-feeding rates and accounted for as much as 75% of the liver's dry weight. This was markedly above the value of 46.4% for the spontaneous FLHS reported earlier (Wolford and Polin, 1972a).

Three other force-feeding experiments by the same authors (Wolford and Polin, 1975) involved feeding lipotropic agents. Although the agents failed to decrease the incidence of FLHS, the dose-response relationship between feed intake and liver hemorrhagic score or liver lipid content was again demonstrated. In one experiment, liver lipids increased from 5.0 g per liver to 18.5 g when 125% force-feeding was used. Liver score increased from 2.0 to 3.9 with FLHS occurring in over 80% of the hens in the 125% group as compared to 12.5% for the controls. The controls varied from 12.2 g of liver fat in the first experiment to 5.0 and 2.6 g for experiments 2 and 3, respectively. Interesting but not explainable was the fact that, as average hepatic lipid in the controls declined, average weight gain increased. The authors indicated that, although excessive energy intake was implicated in general as the cause of FLHS, other factors such as excessive energy retention, lack or presence of specific dietary nutrients or stage of reproduction may be involved. Also, FLHS is not always observed in adult female chickens with very high lipid values or in fatty livers induced by nutritional or toxic substances.

Ivy and Nesheim (1973) found that a wide range of liver fat content can be observed among hens in good production which have

received identical diets. They showed that fat content of livers is markedly influenced by changing energy content of the diet or by force-feeding, but the level of fat in the liver could not be correlated to energy intake. This indicated to them that liver lipid is under metabolic control, independent of energy intake. Their review of the Hartfiel et al. (1970b) work agrees with their own, in that no evidence exists that fatty livers per se are detrimental to laying hen performance. In their work, no mortality was caused by force-feeding, even though the resulting livers were extremely high in fat. They concluded that hens dying from liver hemorrhages have very high liver fat, but apparently aspects other than fat must be present before fatty livers begin to hemorrhage. Genetic susceptibility is possibly a factor, since hens dying from FLHS in the Cornell experimental flock had a close common ancestry.

Wolford and Polin (1972c) subjected hens to restricted feeding (80% of controls) for a period of 6 weeks. The result was a 14 g decrease in liver weight, a decrease in body weight (110% vs. 96% of original weight) and lower abdominal fat (81 vs. 56 g) for restricted compared to ad libitum fed birds. The reduced liver weight was reflected in significantly ($P<.01$) lower moisture, lipid and nonlipid component weights. No liver hemorrhages were noted in the restricted birds. One-half of the restricted birds were kept for an 8-week ad libitum recovery period. The formerly restricted group showed a higher feed consumption rate (10%) than the control ad libitum group, resulting in higher liver lipid percent (37 vs. 32) and total liver

lipid content (5.2 vs. 3.8 g). Higher lipid content in the liver did not necessarily indicate FLHS, yet in some manner lipid predisposed the liver to hemorrhage, since no hemorrhage was observed in livers with less than 3.8 g of lipid. The authors noted that, since liver lipid and hemorrhages were higher in a greater percentage of the previously restricted birds, this appeared to prime the bird for susceptibility to FLHS as a result of a subsequent overeating situation.

Several other reports indicate that energy consumption is a primary factor affecting the level of FLHS (Nesheim et al., 1969; Barton et al., 1966; Barton, 1967; Polin and Wolford, 1973; McDaniel et al., 1959; Wolford and Polin, 1972c). Barton et al. (1966) concluded that protein and energy consumption were the primary factors affecting the development of FLHS, since a low energy, higher fiber ration (14% protein, 2360 kcal ME/kg) prevented its development. Nesheim et al. (1969) proposed feeding lower energy diets in the spring when the weather is warming up to keep hens from a positive energy balance. In the reports by Wolford and Polin (1972c) and Polin and Wolford (1973), they noted that preventing FLHS in laying hens would appear to depend upon preventing obesity. Occasionally putting birds in negative energy balance by either a 24-hour starvation or the use of low energy feed in a less drastic and longer term approach, or simply daily restriction of feed, may be effective methods of decreasing FLHS.

Effect of Grain Source on FLHS

Reports in the literature have indicated that energy level of the diet has an effect on fat accumulation in the liver. Grains vary considerably in energy content and several reports using such energy sources have appeared in the literature.

Jensen et al. (1976e) tested sorghum, corn, triticale, wheat, barley, oats, rye and glucose monohydrate as the major carbohydrate source in laying hen diets for their effect on production and liver lipid parameters. When corn and wheat in various proportions were fed to laying hens, the percent fat and total fat per liver increased as the proportion of corn increased (46 to 60% of dry matter and 11 to 20 g total liver fat, respectively). When comparing different cereal grains in isocaloric diets, the total liver fat accumulated was highest for hens fed sorghum, corn or triticale (14 to 16 g) and lowest for hens fed barley, oats or rye (5 to 7 g). Intermediate levels of fat (10 to 12 g) were obtained with different samples of West Coast wheat. Hens fed diets containing corn had significantly more liver dry matter, percent liver fat and total liver fat per liver than hens fed isocaloric diets containing wheat. No differences in liver parameters were observed in hens fed diets containing either zero, one-half or all glucose monohydrate substituted for corn. Corn and wheat from different geographical locations (Georgia, Midwest or Far West) caused no differences in liver fat. However, considerably more fat accumulated in the livers of hens fed diets containing corn at Washington State University (15.4 to 19.6 g per liver) than in hens fed corn at the University of

Georgia (5.4 to 7.2 g per liver), suggesting the existence of unidentified environmental factors that affect liver fat accumulation.

Two experiments were conducted by Kim et al. (1976) to study the effects of energy source on production criteria and liver fat content. No significant differences were observed for 43-week-old hens on production or liver parameters after 20 weeks on soft white or hard red wheat, triticale or corn diets. In a second experiment, 53-week-old hens were sacrificed after 20 weeks of treatment. Normal and opaque-2 corn-fed hens had significantly higher liver fat than those on a triticale diet. Dietary energy content and body weight were not closely related to liver fat in either experiment.

Two articles have been reported where the effect of brewer's dried grains on production and liver parameters were tested (Jensen et al., 1974a, 1976c). In the first report, two experiments were conducted using distillers dried grains with solubles (DDG/S). Adding 5 or 10% DDG/S improved egg production and appeared to decrease liver weight (6 to 7 g) and liver fat (3 to 10%) in both experiments. However, the results were not always significant. The corn-DDG/S diets resulted in higher liver weight (75 vs. 60 g) and higher liver lipid (63 vs. 47%) than the wheat-DDG/S diets. They suggested that corn either contains or lacks a factor(s) which affects liver lipid metabolism. The difference in energy content of corn and wheat does not account for the difference since the diets were made isocaloric. The authors' second report showed similar results. Liver fat was significantly reduced during the summer experiment when 20% brewer's dried grains was included

in both pelleted and mash diets, below that observed on the 16% basal diet (2.2 vs. 6.2 g). An intermediate response (5.1 g) was obtained with the 10% DDG/S diet. Egg production was not reduced as long as .11% lysine was supplemented. No differences were obtained during the winter experiment.

Polin and Wolford (1976) used the force-feeding technique (150% of normal) to test various carbohydrate sources and corn oil for their effect on liver fat accumulation. They fed the following diets isocalorically: corn-soybean meal control, corn-soybean meal + glucose, corn-soybean meal + corn oil, a purified diet or a corn-oats-wheat bran + corn oil diet. Their data showed that various types of diets and sources of energy in excess can induce FLHS as long as the birds are in positive energy balance. All of the above diets, whether carbohydrate or lipid was the excess source of energy, were equally effective in producing birds with symptoms of FLHS. Their observations allowed for the proposed concept that FLHS in laying hens is the result of a positive energy state over a long enough period of time. They proposed that such a situation accounts for the shift from the usually observed fatty appearance of hepatic tissue in high producing hens to a liver that is fragile or mushy to the touch. This results in weakening to the point of causing blood vessels to rupture from excessive lipid deposits. Therefore, the focus of prevention should be on controlling energy balance. The authors observed that the greatest economic loss would not necessarily be from mortality but from the shunting of excess

energy to carcass lipid, which is later sold as spent hens at a fraction of the value of dietary productive energy.

Effect of Protein Level and Methionine Additions on FLHS

The known relationship of certain amino acids to the biosynthesis of choline in birds has resulted in several investigators studying the effects of amino acid supplementation and protein level on liver fat accumulation. Welch and Couch showed in 1955 that homocystine can be methylated to form methionine in the body of mature fowl, with vitamin B_{12} needed for this methylation. Therefore, the level of protein, or more specifically the level of methionine, may affect FLHS because of its involvement as a methyl group donor in the biosynthesis of choline.

The level of protein in the layer diet has little effect on liver lipids (McDaniel et al., 1959; Owings et al., 1967; Quisenberry et al., 1967). Feeding at several protein levels between 15 and 25% and at various energy levels resulted in no differences in symptoms of FLHS due to protein level. Energy level differences accounted for all differences in the degree of fatty livers.

Supplementing methionine at levels higher than that required for egg production has been shown to contribute to the prevention of FLHS by lowering liver and abdominal fat (Roberson et al., 1970; Griffith et al., 1969). The improvements were not always significant, but feeding choline and vitamin B_{12} with methionine decreased liver fat greater than any one of the nutrients added alone. However, it was not possible in these studies to determine if this reduction was due

to an increased need for the compounds or simply to an increase in the supply of methyl groups.

Since plasma proteins are produced primarily in the liver and are intimately associated with lipid transport to and from lipid depots, Duke et al. (1968) tried to develop an index using plasma protein levels as an indication of developing FLHS. Liver lipid levels of birds in two dietary groups were significantly different from one another. However, the plasma protein levels were not different.

Environmental Factors Affecting the Level of FLHS

Factors that are not directly related to dietary nutrient levels have been shown to affect the accumulation of liver lipid in the laying hen. Water quality is one of those factors that has been suggested as perhaps being of importance in FLHS. Jensen et al. (1976a) collected samples of water from 21 commercial egg producing farms in Georgia with or without a history of FLHS. Water samples from farms with a FLHS history had significantly more calcium, magnesium, strontium, sodium, iron and barium than those reporting no problem. The levels of manganese, boron, copper, zinc and aluminum were not significantly different.

In a similar study, Jensen et al. (1977) obtained water samples from 15 poultry experiment stations that had been classified as to ease or difficulty of producing FLHS in laying hens. Those stations classified as positive had significantly more calcium and cobalt ($P<.05$) and more magnesium, nickel, lead and manganese ($P<.10$) than

those classified as negative. Water from a south Georgia farm that had experienced a high incidence of FLHS was compared in four experiments with the University of Georgia water (UGA, a negatively classified water). Hens in floor pens and cages or pullets raised from 1 day of age that received the south Georgia water did not accumulate more liver fat than hens on the UGA water. The birds were sacrificed at 48 weeks of age. In addition, adding calcium or magnesium at 100 and 50 ppm, respectively, did not increase liver lipids. Although the quantities of calcium and magnesium in water differed significantly between various stations, it was difficult to comprehend how such differences could play a significant role in lipid metabolism. One possibility, however, may be that calcium and magnesium in the water can play a role by interfering in absorption of some of the trace elements.

Environmental temperature has been shown to affect liver lipid accumulation. Griffith et al. (1969) indicated in their work that liver lipid levels peaked during the hot summer months. Wolford's subsequent work (1971) agreed with the observations of Griffith et al. Placing hens in a temperature controlled room at 1.7 C for 28 days resulted in significantly lower ($P < .01$) total liver lipid as compared to those housed at 26.7 C (9.0 vs. 20.8 g per 100 g wet liver weight).

Price et al. (1957) noted that liver lipids accumulate to a larger degree in birds housed in cages as compared to birds on litter. Lipid content averaged 30% (dry matter basis) for the floor producing birds while averaging from 27 to 53% in cages, depending on the diet fed. Liver weights were comparable in laying hens on litter or in

cages (40 to 50 g). However, nonlayers in cages suffering from FLHS had livers weighing in the range of 58 to 71 grams. The authors indicated a possible deficiency in the cage layer diet or that the higher fat levels were due to a restriction in bird movement.

Several reports have since agreed with Price et al. (1957) that cage type systems promote the incidence of FLHS, while there is little incidence in birds that are allowed to exercise. Griffith et al. (1969), Barton et al. (1966), Barton (1967) and Hartfiel et al. (1970a,b, as reviewed by Ivy and Nesheim, 1973) observed that hens in cages had higher liver fat content than those in floor pens. In addition, Hartfiel et al. (1970a) showed that forced exercise and consumption of feces caused a reduction of liver fat in hens housed in cages.

Housing costs often force commercial producers to increase bird density above recommended levels. Normal mortality during the laying cycle decreases the number of birds per cage. Owings et al. (1967) and Jensen et al. (1976d) studied the effects of bird density in cages on fat accumulation in the liver. Owings et al. (1967) found no difference in the distribution of fatty acids or amount of liver lipids between birds housed two or three per cage (25 x 41 cm) at three protein levels (16, 17.5 and 19%).

Jensen et al. (1976d) housed birds in similar size cages (25 x 46 cm) at one or three birds per cage. Hens housed individually laid significantly more eggs (7%), consumed significantly more feed (113 vs. 102 g) and had significantly larger livers (57 vs. 52) with a higher

lipid content (39.9 vs. 34.7% of dry matter or 9.5 vs. 6.6 g per liver) than those housed three per cage. Body weight was significantly higher for individually caged birds in one experiment but not in another. No differences in egg weight or feed efficiency were observed. In a second experiment, hens socially dominant in the three-bird pens had higher liver fat than those lower in the peck order.

Effect of Bird Strain on the Incidence of FLHS

Genetic susceptibility to FLHS is difficult to ascertain because most researchers do not indicate which strain of hens were used in their research. Two articles have appeared in the literature which have reported studies testing for strain susceptibility to FLHS. Jensen et al. (1976d) found no difference in fat accumulation between Babcock B-300 and DeKalb 171 strains in their cage density work. In work by Garlich et al. (1975), liver lipid values were determined for 20 varieties of 71-week-old laying hens managed in three different confinement systems. Means ranged from 25.8 to 49.0% liver lipid (dry matter basis) with hens two per cage having significantly higher lipid (40.1%) than seven per cage (36.6%) or the floor confinement system (37.8%). They considered a satisfactory range of lipid values for hens this age to be between 25 and 49%, since none of the groups developed FLHS during the laying year.

Hormonal Control of Liver Fat and Histological Observations of Fatty Livers

Hepatic lipid metabolism is considered to be at least partially controlled by hormonal levels because hens out of production have low

hepatic lipid levels. Therefore, several researchers have tested dietary additions of hormone compounds for their effects on FLHS. Roberson et al. (1970) found that thiouracil and diensestrol di-acetate promoted a fatty liver condition, while thyroprotein contributed to the prevention of liver and abdominal lipid accumulation.

Wolford (1971) and Wolford and Polin (1975) fed a thyroid stimulator in the form of iodinated casein. In the first report, feeding .033% iodinated casein to laying hens for 23 weeks resulted in a reduction in liver lipid ($P < .01$) by one-half (12.2 vs. 23.6 g per 100 g wet liver weight). No effect on liver weight was indicated. In the later report, however, iodinated casein failed to decrease liver lipid in a force-feeding type experiment.

Harms et al. (1977) experimentally produced FLHS by injecting estradiol (12 mg) 3 days prior to sacrifice or by feeding 5000 ppm potassium iodide (KI). Liver weight increased by about 25% with either treatment and up to 50% when both treatments were given. This was true whether based on absolute weight or percent of body weight. Total grams of liver fat increased from 4.8 g in the controls to 6.8, 8.5 and 7.8 in the KI, estradiol and combination treatments, respectively. This increase, however, did not affect fatty acid composition of the lipid.

The histological appearance of the livers for the control and KI birds was similar with a majority of the hepatic cells containing lipid droplets of small or moderate size. Livers from the estradiol-treated birds usually contained large lipid droplets which caused pronounced swelling of involved cells. Some focal areas of necrosis were seen.

The combination of KI and estradiol caused more advanced lesions with diffuse hemorrhagic necrosis. These histological observations agree with those reported to be typical of FLHS by Wolford et al. (1971) and Wills and Savage (1974). Harms et al. (1977) suggested that feeding KI and estradiol can be used as a model for studying FLHS.

Wills and Savage (1974) reported that livers showing both hemorrhages and high fat infiltration contained numerous large non-membrane bound lipid droplets within the liver cell. On analysis, the excess lipid was high in triglycerides when compared to the level of structural lipids. Mitochondria were less numerous and poorly defined as compared to normal livers.

Wolford et al. (1971) reported on liver sections from "normal" laying hens. They found the cells to lie in cord-like rows radiating from a central vein. Those with FLHS were distended due to fat infiltration and appeared to lack the normal cord-like organization. Fat infiltration forced the cell nucleus to the side of the cell instead of being in the center. Staining with oil red O showed normal livers to have fat droplets that were intracellular, while FLHS inflicted livers showed an abundance of intracellular fat and some extracellular lipid as well.

A Review of a Similar Condition in Broilers - Fatty Liver-Kidney Syndrome

Fatty liver-kidney syndrome (FLKS) has been reported to cause significant mortality among broiler type chicks in the United Kingdom (Blair and Whitehead, 1974), Australia (Payne et al., 1974) and Denmark (Marthedal et al., 1974). Normal mortality from this disease is about

1 to 2% but can run as high as 10 to 20%. Birds are most susceptible during the 3- to 4-week-old stage, but it can occur as early as 4 days and as late as 8 weeks of age. Some suggested causes of the fatal disease have been nutritional, environmental, genetic and toxicological.

Upon death, the birds are usually found on their sternum with head extended. The liver is pale and swollen, with hemorrhages on the periphery of the lobes. The heart is pale and the adipose tissue often has a pinkish cast. The kidneys are swollen and often pale. A blackish fluid of unknown significance is usually found in the crop, gizzard and duodenal loop. Abnormal deposition of lipid occurs in many organs including the liver, heart, kidneys, alimentary tract, muscle fibers, central nervous system, thyroid, pancreas and adrenal medulla.

Blair and Whitehead (1974) found FLKS to show a twofold increase in lipid in the liver and kidney with triglycerides accounting for most of the increase. This agrees with the work of Wills and Savage (1974) with laying hens. Palmitoleic acid increased significantly at the expense of stearic acid. This is in agreement with the work of Roland and Edwards (1971). The work of Blair and Whitehead (1974) also showed protein level of the diet to be inversely correlated to the incidence of FLKS, while the addition of the lipotropic agents methionine, choline, vitamin B_{12} and selenium showed no clear involvement. Mortality was found to be higher with barley and corn diets than with wheat diets, while increasing dietary fat level from 2 to 7% decreased mortality from the disease from 23 to 5%. Corn oil, tallow or olive oil produced similar reductions.

In Blair and Whitehead's (1974) work, a diet of 69% wheat, 18% field beans, 4% soybean meal and 6.3% herring meal plus vitamins and minerals (2850 kcal/kg, 18% protein and 1.9% fat) was found to consistently produce 15% mortality from FLKS. The calorie-protein ratio was subsequently found to be important. Diets with ratios of 130 and 167 kcal ME/kg produced mortality of .9% and 19%, respectively. Increasing the house temperature by 3 C over the recommended temperatures increased mortality by as much as 20%.

Further investigation by Blair and Whitehead (1974), using the above diet with and without various vitamins, showed that supplements of .36 mg/kg of biotin decreased FLKS mortality from 23.0 to .2%. This was further verified by _in vitro_ liver studies, since a failure of gluconeogenesis in affected bird livers was corrected by the addition of biotin to the homogenate. Another interesting aspect was the decrease in dermal lesions when the above diet was supplemented with fat, indicating an interaction between fat and biotin in this diet.

The work by Payne _et al._ (1974) indicated that feeding 145 µg of biotin per kg of diet in a wheat-meal diet resulted in maximum growth and the elimination of mortality due to FLKS. Mortality dropped from as high as 24% due to FLKS to 0% when biotin was added.

Marthedal _et al._ (1974) indicated in their report that the disease has decreased since the late 1950's. They attributed this decrease to the application of feeds with higher contents of energy and protein.

Jensen et al. (1976b) and Chah et al. (1975) found biotin supplementation to be of no benefit in reducing hepatic liver lipid in the laying hen. In addition, analysis of the fatty acids in livers from hens dying of fatty liver syndrome failed to reveal any marked increase in palmitoleic acid (Ivy and Nesheim, 1973). The available evidence to date thus indicates that the two syndromes are different in etiology, one being prevented and the other not affected by biotin supplementation.

MATERIALS AND METHODS

Two types of experiments were used to evaluate several nutritional aspects in this study with caged laying hens. Experiments 1 through 4 were ad libitum type experiments, while in experiments 5 through 11, a force-feeding technique similar to the one devised by Wolford and Polin (1972b) was utilized to increase the incidence of the disease.

Ad Libitum Experiments

Experiment 1. Two hundred eighty-eight 24-week-old pullets of three commercial strains that had been raised on one of two grower diets were randomly allotted at four birds per wire cage (61 x 41 cm). The three strains of birds used in this experiment were Babcock B-300, Shaver Starcross 288 and Hyline W-36. The pullets had been started as chicks in a cage system and were given a standard 20% protein starter ration until 10 weeks of age. This was followed with either a 10 or 12% protein grower diet (Table 1) until 20 weeks of age. The birds had then been transferred to the laying house.

Table 2 shows the composition of the layer diets that were fed for 12, 28-day production periods. Choline and/or biotin were supplemented (1500 and 1.1 mg/kg, respectively) in a 14% protein, 10% fat, control layer diet to give four treatments, each being replicated three times for a total of 72 experimental units.

Data were recorded daily for each cage and analyzed statistically by 28-day periods. All birds were sacrificed by cervical dislocation

Table 1. Composition of Grower Diets in Experiments 1 and 5

Ingredients	Treatment 1 %	Treatment 2 %
Ground yellow corn	47.4	5.3
Ground oats	43.8	62.0
Reground oat hulls	--	27.0
Soybean meal (48%)	5.2	2.4
Limestone	1.5	1.5
Dicalcium phosphate	.6	.6
Trace mineral salt[a]	.5	.5
Vitamin premix[b]	.5	.5
DL-methionine	.06	.07
L-lysine	.21	.15
Calculated analysis		
Crude protein (%)	11.8	10.3
ME (kcal/kg)	2814	1907
Lysine (%)	.63	.49
Methionine (%)	.32	.24

[a] Trace mineral salt. Contains in percent: Zn, .35; Mn, .20; Fe, .20; Mg, .15; Cu, .03; Co, .005; I, .007; NaCl, 97.0.

[b] Vitamin premix. Contains per kg: vitamin A, 21,000 IU; vitamin D_3, 56,800 IU; vitamin E, 910 IU; vitamin B_{12}, .8 mg; riboflavin, 275 mg; niacin, 1800 mg; d-pantothenic acid, 365 mg; choline, 16 g; menadione, 45 mg, folic acid, 100 mg; d-biotin, 4.5 mg; ethoxyquin, 10.0 g.

Table 2. Composition of Layer Diets in Experiments 1 and 5

Ingredient	Control[a] %
Ground yellow corn	63.7
Soybean meal (48%)	16.2
Alfalfa meal (17%)	2.0
Yellow grease	10.0
Limestone	5.0
Dicalcium phosphate	2.0
Trace mineral salt[b]	.5
Vitamin premix[c]	.5
DL-methionine	.1
Calculated analysis	
Crude protein (%)	13.9
ME (kcal/kg)	3297
Lysine (%)	.69
Methionine (%)	.35

[a] Treatment 2 = as control + 1500 mg choline per kg. Treatment 3 = as control + 1.1 mg biotin per kg. Treatment 4 = as 2 + 1.1 mg biotin per kg.

[b] See Table 1.

[c] Vitamin premix. Contains per kg: vitamin A, 21,000 IU; vitamin D_3, 56,800 IU; vitamin E, 910 IU; vitamin B_{12}, .8 mg; menadione, 45 mg; folic acid, 100 mg; ethoxyquin, 10.0 g; riboflavin, 275 mg; niacin, 1800 mg; d-pantothenic acid, 365 mg.

at 72 weeks of age. Triplicate samples of livers pooled by cage were analyzed for lipid content by drying in an oven at 100 C for 20 hours and then refluxing with ethyl ether in a soxhlet extraction apparatus.

All data were analyzed statistically by least squares analysis of variance (Steel and Torrie, 1960) and Dunnett's (1955) multiple comparison procedure was used where applicable.

Experiment 2. Three hundred sixty 24-week-old pullets of three commercial strains (Babcock B-300, DeKalb 231 and Hyline W-36) were randomly allotted into groups of 12 (one of each strain per cage) with three birds per cage (30 x 41 cm). The birds had been raised on standard corn-soybean starter (20% protein) and grower diets (12% protein) in a growing cage system.

Table 3 shows the composition of the layer diets that were fed for 13, 28-day production periods. Corn, oats and wheat diets of about 14% protein were fed with 2 and 5% added levels of fat to give a total of six layer diets. Each experimental unit was replicated five times for a total of 30 units.

Production data were recorded daily for each group of four cages (12 birds) and analyzed statistically by 28-day periods. Three birds from each group (one of each strain in reproductive condition) were sacrificed by cervical dislocation at 76 weeks of age. Livers were removed, scored for hemorrhages (1 = no hemorrhages, 2 = 1 to 10 hemorrhages, 3 = 10 to 25 hemorrhages and 4 = more than 25 hemorrhages) and weighed individually. They were then analyzed individually for lipid content as described in Experiment 1. The liver data were

Table 3. Composition of Layer Diets Used in Experiment 2

Ingredients	Treatments					
	1	2	3	4	5	6
	%	%	%	%	%	%
Ground yellow corn	73.5	70.5	--	--	--	--
Ground oats	--	--	79.0	76.0	--	--
Ground wheat	--	--	--	--	86.0	83.0
Soybean meal (48%)	14.4	14.4	8.9	8.9	2.0	2.0
Alfalfa meal (17%)	2.0	2.0	2.0	2.0	2.0	2.0
Yellow grease	2.0	5.0	2.0	5.0	2.0	5.0
Limestone	5.0	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0
Trace mineral salt[a]	.5	.5	.5	.5	.5	.5
Vitamin premix[b]	.5	.5	.5	.5	.5	.5
DL-methionine	.1	.1	.2	.2	.2	.2
L-lysine	--	--	--	--	.2	.2
Calculated analysis						
Crude protein (%)	13.9	13.6	14.0	13.4	14.2	13.8
ME (kcal/kg)	2971	3099	2431	2573	2915	3019
Lysine (%)	.66	.65	.59	.54	.58	.56
Methionine (%)	.36	.35	.42	.41	.37	.42

[a] See Table 2.
[b] See Table 1.

separated out by strain and statistically analyzed for layer diet, strain of birds and dietary fat level. This could not be done for the production data. All data were analyzed statistically by the procedure given in Experiment 1.

Experiment 3. Seventeen hundred twenty-eight pullets, raised (from 10 to 20 weeks) on one of four grower diets in a cage system (Table 4), were randomly distributed four birds per cage (41 x 41 cm). Groups of three cages (12 birds) were fed 16 or 12% layer diets as shown in Table 5. Three strains of birds (Babcock B-300, DeKalb 231 and Hyline W-36) were used in this experiment and each experimental unit was replicated six times for a total of 144 units.

Production data were recorded daily for each experimental unit and analyzed statistically by 28-day periods. Seventy-two birds (in reproductive condition) were randomly selected and sacrificed by cervical dislocation at 76 weeks of age. Livers were removed, scored for hemorrhages (as described in Experiment 2) and weighed individually. They were analyzed individually for lipid content as described in Experiment 1. Production and liver data were analyzed statistically by the procedure given in Experiment 1.

Experiment 4. Two hundred forty 20-week-old pullets of two strains were randomly allotted six birds per cage (61 x 41 cm). One strain was a commercial strain (Babcock B-300), while the other was of the Regional Control SCWL straightbred research stock. The birds had been raised on standard corn-soybean starter (20% protein) and grower

Table 4. Composition of Grower Diets Used in Experiment 3

Ingredients	Treatments	
	1 to 3[a]	4
	%	%
Ground yellow corn	50.0	30.0
Ground oats	30.0	--
Dehulled oats	--	50.0
Soybean meal (48%)	4.0	--
Alfalfa meal (17%)	10.0	10.0
Yellow grease	2.0	2.0
Dicalcium phosphate	2.0	2.0
Limestone	1.0	1.0
Trace mineral salt[b]	.5	.5
Vitamin premix[c]	.5	.5
Calculated analysis		
Crude protein (%)	11.6	12.8
ME (kcal/kg)	2828	3076
Lysine (%)	.42	.37
Methionine (%)	.21	.19

[a] Treatment 2 = as 1 + .13% DL-methionine. Treatment 3 = as 2 + .27% L-lysine.
[b] See Table 2.
[c] See Table 1.

Table 5. Composition of Layer Diets Used in Experiment 3

Ingredients	Treatments	
	16% protein	12% protein
	%	%
Ground yellow corn	66.0	81.0
Soybean meal (48%)	20.0	9.0
Alfalfa meal (17%)	2.0	2.0
Limestone	5.0	5.0
Dicalcium phosphate	2.0	1.5
Trace mineral salt[a]	.5	.5
Vitamin premix[b]	.5	.5
DL-methionine	--	.15
L-lysine	--	.20
Calculated analysis		
Crude protein (%)	15.9	12.0
ME (kcal/kg)	3317	2933
Lysine (%)	.81	.70
Methionine (%)	.28	.38

[a] See Table 2.
[b] See Table 1.

diets (12% protein) in a grower cage system. They were maintained on a 16% layer diet (Table 5, Diet 1) until 28 weeks of age, at which time the experiment was begun.

Table 6 shows the composition of the layer diets that were fed for nine, 28-day periods. Diets containing cellulose, oat hulls or wheat bran were made isocaloric to a 14% protein corn-soybean meal diet and fed to test their ability to suppress liver lipids. The three experimental diets were made equal in methionine, lysine and fiber content. Each experimental unit was replicated five times for a total of 40 units.

Production data were recorded daily for each cage and analyzed statistically by 28-day periods. Two birds from each cage, that were in reproductive condition, were sacrificed by cervical dislocation at 64 weeks of age. The livers were removed, scored for hemorrhages (as described in Experiment 2) and weighed individually. They were then analyzed individually for lipids by the procedure described in Experiment 1. Production and liver data were analyzed statistically by the procedure given in Experiment 1.

Force-Fed Experiments

Experiment 5. Seventy-two 20-week-old pullets of three commercial strains that had been raised on one of two grower diets were randomly allotted (three per treatment) to individual cages (20 x 41 cm). The three strains of birds used in this experiment were Babcock B-300, Shaver 288 and Hyline W-36. The pullets had been started as

Table 6. Composition of Layer Diets Used in Experiments 4 and 11

Ingredients	Treatments 1 %	2 %	3 %	4 %
Ground yellow corn	73.5	71.2	60.0	54.8
Soybean meal (48%)	14.5	14.8	15.0	14.0
Alfalfa meal (17%)	2.0	2.0	2.0	2.0
Yellow grease	2.0	2.0	7.0	6.4
Limestone	5.0	5.0	5.0	5.0
Dicalcium phosphate	2.0	2.0	2.0	2.0
Trace mineral salt[a]	.5	.5	.5	.5
Vitamin premix[b]	.5	.5	.5	.5
Solka Floc[c]	--	2.0	--	1.2
Oat hulls	--	--	8.0	--
Wheat bran	--	--	--	10.0
DL-methionine	.1	.1	.1	.1
Calculated analysis				
Crude protein (%)	13.9	13.7	13.3	13.7
ME (kcal/kg)	2971	2955	2985	2957
Lysine (%)	.65	.66	.65	.66
Methionine (%)	.36	.35	.36	.35
Fiber (%)	2.8	4.7	4.6	4.7

[a] See Table 2.
[b] See Table 1.
[c] Solka Floc, BW-20, Brown Company, Berlin, New Hampshire, 03570.

chicks in a grower cage system and were given a standard 20% starter ration until 10 weeks of age. They were then fed either a 10 or 12% protein diet (Table 1) until 20 weeks of age. At this time, the birds were transferred to the laying house.

Table 2 shows the composition of the layer diets that were fed until the termination of the experiment at 32 weeks of age. These are the same diets that were fed ad libitum in Experiment 1. Force-feeding was begun at 27 weeks of age and continued for 5 consecutive weeks. Three days of force-feeding were missed during the course of the experiment. For this reason, the birds were kept on the force-feeding regime longer than the normal 21 days required to produce FLHS as recommended by Wolford and Polin (1972b). Birds were allowed to consume additional feed ad libitum while being force-fed.

Special preparation of the diet for Experiments 5 through 11 was required in order for it to be force-fed. A mixture of approximately 1 part diet and 1.5 parts tap water (weight to volume) was homogenized in a blender for about 2 minutes to a consistency of "cake batter." In Experiments 10 and 11, the diets were run through a Wiley mill first to help avoid extreme wear on the blender. As a result, the required blending time was reduced considerably. The diet to water ratio had to be adjusted on some diets that were tested in these experiments because diet density varied considerably.

Following blending, the mixture was forced directly into the crop by means of a 60 cc polypropylene syringe through a .93 cm diameter

"Tygon" tube. Force-feeding was performed two times each day at approximately 12-hour intervals.

At the end of the experiment, all hens were sacrificed by cervical dislocation. Livers were removed, evaluated for hemorrhages (as described in Experiment 2) and weighed individually. Hepatic lipid was determined on individual livers by the method described in Experiment 1.

Production data had been recorded daily for individual hens, and these and the liver data were analyzed statistically by the procedure given in Experiment 1.

Experiment 6. Thirty 27-week-old hens of the commercial strain DeKalb 231 were randomly allotted (five per treatment) to individual cages (20 x 41 cm). The birds had been grown to 20 weeks of age under the standard conditions described in Experiment 2. The birds were then placed on a 16% protein layer diet (Table 5, Diet 1) until the start of the experiment.

Table 7 shows the composition of the layer diets that were fed for the duration of the 21-day experiment. Corn and wheat were compared in 14% protein diets to test their effects on liver lipid accumulation. Attempts were made to feed the two diets at approximately 120 and 140% of ad libitum feeding to give six treatments. Force-fed birds were allowed to consume additional feed ad libitum. Both diets were prepared and force-fed by the method described in Experiment 5. Production data were recorded daily during the course of the experiment.

Table 7. Composition of Layer Diets Used in Experiment 6

Ingredients	Treatments 1	2
	%	%
Ground yellow corn	73.5	--
Ground wheat	--	86.0
Soybean meal (48%)	14.4	2.0
Alfalfa meal (17%)	2.0	2.0
Yellow grease	2.0	2.0
Limestone	5.0	5.0
Dicalcium phosphate	2.0	2.0
Trace mineral salt[a]	.5	.5
Vitamin premix[b]	.5	.5
DL-methionine	.1	.1
L-lysine	--	.2
Calculated analysis		
Crude protein (%)	13.9	14.2
ME (kcal/kg)	2971	2915
Lysine (%)	.66	.58
Methionine (%)	.36	.37

[a] See Table 2.
[b] See Table 1.

At 30 weeks of age (after 21 days of continuous force-feeding), all hens were sacrificed by cervical dislocation. Livers were removed, evaluated for hemorrhages (see Experiment 2) and weighed individually. Hepatic lipid was determined on individual livers by the method described in Experiment 1. Production and liver data were then analyzed statistically by the procedure given in Experiment 1.

Experiments 7 and 8. Thirty hens, in both experiments, of the commercial strain Shaver 288 were randomly allotted (five per treatment) to individual cages (20 x 41 cm). The hens were maintained to 30 and 48 weeks of age under the standard conditions described in Experiment 2. At 20 weeks of age, the birds had been placed on a 16% protein layer diet (Table 5, Diet 1) until the start of the force-feeding experiment.

Table 8 shows the composition of the layer diets that were fed for the duration of both 21-day experiments. Fat additions of 2, 5 and 8% were compared in isocaloric corn-soybean meal diets. In Experiment 7, attempts were made to force-feed the birds at 125% of the ad libitum fed birds. Force-fed birds were allowed to consume additional feed ad libitum. In Experiment 8, the control treatments were restricted to 100 g of feed per day, while the force-fed birds were given 125 g per day with no additional ad libitum feeding allowed. The three diets were prepared and force-fed by the method described in Experiment 5. Production data were recorded daily during the course of the experiment.

At 33 and 51 weeks of age (Experiments 7 and 8, respectively), all hens were sacrificed by cervical dislocation. Livers were removed,

Table 8. Composition of Layer Diets for Experiments 7 and 8

Ingredients	Treatments 1 %	2 %	3 %
Ground yellow corn	73.5	65.0	56.4
Soybean meal (48%)	14.5	15.5	17.0
Alfalfa meal (17%)	2.0	2.0	2.0
Yellow grease	2.0	5.0	8.0
Limestone	5.0	5.0	5.0
Dicalcium phosphate	2.0	2.0	2.0
Trace mineral salt[a]	.5	.5	.5
Vitamin premix[b]	.5	.5	.5
Solka Floc[c]	--	4.5	8.5
DL-methionine	.1	.1	.1
Calculated analysis			
Crude protein (%)	13.9	13.9	13.9
ME (kcal/kg)	2971	3093	3107
Lysine (%)	.66	.67	.69
Methionine (%)	.36	.35	.34

[a] See Table 2.
[b] See Table 1.
[c] See Table 6.

evaluated for hemorrhages (See Experiment 2) and weighed individually. Hepatic lipid was determined on individual livers by the method described in Experiment 1. Production and liver data were then analyzed statistically by the procedure given in Experiment 1.

Experiment 9. Fifty-six 66-week-old hens of the commercial strain Shaver Starcross 288 were randomly allotted (seven per treatment) to individual cages (20 x 41 cm). They had been grown to 20 weeks of age under the standard conditions described in Experiment 2. At 20 weeks of age, the birds were placed on a 16% protein layer diet (Table 5, Diet 1) until the start of the force-feeding experiment.

Table 9 shows the composition of the layer diets that were fed for the duration of the 21-day experiment. Treatment 1 was the basal corn-soybean meal diet (14% protein) used as the control in several other experiments reported here. Additions of 1500 mg choline per kg of diet and/or .1% additional DL-methionine resulted in the four dietary treatments used. Attempts were made to force-feed at 125% of the ad libitum fed birds. The four diets were prepared and force-fed by the method described in Experiment 5. Production data were recorded daily during the course of the experiment.

At 69 weeks of age, all hens were sacrificed by cervical dislocation. Livers were removed, evaluated for hemorrhages (see Experiment 2) and weighed individually. A sample of each liver was fixed in 10% formalin for paraffin mounting and each was sectioned and stained with hematoxylin nuclear and eosin cytoplasmic stain. Hepatic

Table 9. Composition of Diets Used in Experiment 9

Ingredients	Control[a] %
Ground yellow corn	73.5
Soybean meal (48%)	14.4
Alfalfa meal (17%)	2.0
Yellow grease	2.0
Limestone	5.0
Dicalcium phosphate	2.0
Trace mineral salt[b]	.5
Vitamin premix[c]	.5
DL-methionine	.1
Calculated analysis	
Crude protein (%)	13.9
ME (kcal/kg)	2971
Lysine (%)	.66
Methionine (%)	.36

[a] Treatment 2 = as control + 1500 mg choline per kg. Treatment 3 = as control + .1% additional DL-methionine. Treatment 4 = as 3 + 1500 mg choline per kg.

[b] See Table 2.

[c] See Table 1.

lipid was determined on individual livers by the method described in Experiment 1. Production and liver data were then analyzed statistically by the procedure given in Experiment 1.

Experiment 10. Forty 73-week-old hens of the commercial strain Shaver Starcross 288 were randomly allotted (five birds per treatment) to individual cages (20 x 41 cm). They had been grown to 20 weeks of age under the standard conditions described in Experiment 2. At 20 weeks of age, the birds were placed on a 16% protein layer diet (Table 5, Diet 1) until the start of the force-feeding experiment.

Table 10 shows the composition of the layer diets that were fed for the duration of the 21-day experiment. Four dietary treatments were used consisting of corn-soybean meal or oats-soybean meal with or without supplemental choline. Attempts were made to feed the force-fed treatments at about 125% of the ad libitum fed birds. The four diets were prepared and force-fed by the method described in Experiment 5. Production data were recorded daily during the course of the experiment.

At 76 weeks of age, all hens were sacrificed by cervical dislocation. Livers were removed, evaluated for hemorrhages (see Experiment 2) and weighed individually. Two livers from each treatment were sampled for histological observation. One sample was fixed in 10% formalin for paraffin mounting, while duplicate samples were mounted using a freezing method (Humason, 1972). Hematoxylin nuclear stain and eosin cytoplasmic stain were used in both procedures. In addition, oil red O was used in the freeze method to stain any lipid that was present.

Table 10. Composition of Layer Diets Used in Experiment 10

Ingredients	Treatments[a] 1	2
	%	%
Ground yellow corn	73.5	--
Ground oats	--	79.5
Soybean meal (48%)	14.4	8.5
Alfalfa meal (17%)	2.0	2.0
Yellow grease	2.0	2.0
Limestone	5.0	5.0
Dicalcium phosphate	2.0	2.0
Trace mineral salt[b]	.5	.5
Vitamin premix[c]	.1	.1
DL-methionine	.1	.1
Calculated analysis		
Crude protein (%)	13.9	14.0
ME (kcal/kg)	2971	2431
Lysine (%)	.66	.59
Methionine (%)	.36	.32

[a] Treatment 3 = as 1 + 1500 mg choline per kg. Treatment 4 = as 2 + 1500 mg choline per kg.
[b] See Table 2.
[c] See Table 1.

Hepatic lipid was determined on individual livers by the method described in Experiment 1. Production and liver data were then analyzed statistically by the procedure given in Experiment 1.

Experiment 11. Forty 65-week-old hens of the straightbred Regional Control SCWL stock were randomly allotted (five per treatment) to individual cages (20 x 41 cm). They had been grown under standard conditions to 20 weeks of age as described in Experiment 2. At 20 weeks of age, the birds were placed on a 16% protein layer diet (Table 5, Diet 1) until the start of the force-feeding experiment.

Table 6 shows the composition of the layer diets that were fed for 2 weeks prior to and for the duration of the 21-day experiment. These are the same diets described and used for the ad libitum study labeled Experiment 4. The four diets were prepared and force-fed by the method described in Experiment 5. Production data were recorded daily during the course of the experiment.

At 68 weeks of age, all hens were sacrificed by cervical dislocation. Livers were removed, evaluated for hemorrhages (see Experiment 2) and weighed individually. Duplicate samples were taken of two livers, one considered to be a normal liver and the other a severe case of FLHS. The two samples were fixed in both glutaraldehyde and 10% formalin. Microthin sections were made for electron microscopic observation.

Hepatic lipid was determined on individual livers by the method described in Experiment 1. Production and liver data were then analyzed statistically by the procedure given in Experiment 1.

RESULTS AND DISCUSSION

Ad Libitum Experiments

Experiment 1. Average production data and the analysis of variance for egg production, feed consumption, egg weight and feed efficiency are shown in Tables 11 and 12, respectively. Hen-day production was considered to be quite low, averaging about 62%. This could be attributed to the high energy and relatively low protein level that was used to try to increase the incidence of FLHS. Supplementing choline and/or biotin at about twice the N.R.C. (1971) recommended levels resulted in an enhancement of egg production and feed efficiency due to choline, while biotin had little effect. The decrease in egg size from hens on the biotin treatment is unexplainable. Pullets grown on a 10% protein diet produced more eggs than those grown on a 12% diet. Strain of bird caused no significant differences with the exception of 1 or 2 g differences in egg weight. Several interactions occurred between treatments which probably prevented several of the treatment effects from being significantly different.

The beneficial effects shown here due to choline are in agreement with those of other researchers. Although reports are somewhat conflicting, Welch and Couch (1955), Soloma et al. (1965), Holmes and Kramer (1965), Schexnailder and Griffith (1973), Griffith and Schexnailder (1972) and Griffith et al. (1969) showed increases in egg production when supplementing choline to a practical diet.

Treatment data, showing effects on final body weight and liver parameters, are shown in Tables 13 and 14. Several significant trends

Table 11. Effect of Strain and Grower and Layer Diets on Production Parameters--Experiment 1[a,b]

Treatment	Hen-day production	Hen-day feed consumed	Egg weight	Feed per dozen eggs
	%	g	g	kg
Layer diet[c]				
Control	59.0	93.8	59.3	1.96
+ choline	66.5A	94.3	61.3	1.73
+ biotin	56.8	88.1	70.0a	2.07
+ choline and biotin	66.1A	91.8	60.3	1.66a
Strain				
Babcock B-300	61.9	96.0	60.9	2.00
Shaver 288	63.3	91.6	59.3a	1.81
Hyline W-36	61.1	88.3	58.3a	1.76
Grower diet[d]				
12%	59.6B	91.6	59.7	1.97A
10%	64.6A	92.4	59.3	1.74B

[a] Average for 12, 28-day periods.

[b] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer and strain effects, Dunnett's t was used to test for differences from control or the highest value, respectively.

[c] See Table 2 for diet composition.

[d] See Table 1 for diet composition.

Table 12. Analysis of Variance for Production Parameters in Experiment 1

Source of variation	d.f.	Mean squares			
		Hen-day production	Hen-day feed consumed	Egg weight	Feed per dozen eggs
Replicates	2	1858.4	7499.1	27.55	.964
Grower diet	1	5270.3**	144.1	21.60	10.980**
R x D	2	9.3	139.0	63.59	.268
Strains	2	352.6	4319.3	484.36*	4.479
R x S	4	435.3	714.8	43.12	2.150
D x S	2	487.6*	1344.2**	114.25	1.593
R x D x S	4	66.3	30.5	72.92	.751
Treatment	3	5273.7**	1697.7	722.42**	7.781*
R x T	6	529.2	864.7	68.50	1.112
D x T	3	1464.3	1373.0	160.28*	4.118*
R x D x T	6	797.5	1131.8	21.42	.544
S x T	6	945.0	454.4	40.81	2.746
R x S x T	12	600.3	1050.7	58.95	1.192
D x S x T	6	934.6	227.9	31.43	2.684
RDST	12	555.9	1007.2	43.10	2.159
Months	11	2254.4**	6816.1**	812.80**	7.342**
R x M	22	156.2	283.3	9.08	.395
D x M	11	80.0	30.2	5.91	.464
R x D x M	22	153.8	166.2	16.71	.376
S x M	22	400.1**	214.8	23.75*	.713
R x S x M	44	91.1	144.6	9.44	.465
D x S x M	22	241.8	175.1	11.83	.539
RDSM	44	125.1	107.3	15.99	.397
T x M	33	257.7**	99.5	42.68**	.814
R x T x M	66	102.6	143.4	16.94	.327
D x T x M	33	142.8	173.6	14.83	.678
RDTM	66	162.7	124.3	14.59	.430
S x T x M	66	79.4	70.7	16.89	.407
RSTM	132	114.8	97.9	12.24	.476
DSTM	66	118.1	101.6	14.97	.468
RDSTM	132	132.3	98.4	14.48	.515

* $P < .05$.
** $P < .01$.

Table 13. Effect of Strain and Grower and Layer Diets on Several Liver Parameters in Experiment 1[a,b]

Treatment	Liver weight	Liver lipid wet basis	Total liver lipid	Liver as part of body weight	Final body weight
	g	%	g	%	kg
Layer diet[c]					
Control	60.3	21.0	13.3	3.25	1.86
+ choline	51.3A	12.6A	6.7A	2.68A	1.91
+ biotin	54.4a	17.7	9.9a	3.09	1.78
+ choline and biotin	46.9A	11.1A	5.2A	2.58A	1.82
Strain					
Babcock B-300	56.9	17.0	10.5	3.07	1.86
Shaver 288	54.2	14.5	8.2	2.98	1.83
Hyline W-36	48.7A	15.3	7.7	2.65A	1.83
Grower diet[d]					
12%	53.0	15.4	8.8	2.86	1.86
10%	53.5	15.8	8.8	2.94	1.83

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer and strain effect Dunnett's t was used to test for differences from control or the highest value, respectively.

[b] Hens were 72 weeks of age when sacrificed.

[c] See Table 2 for diet composition.

[d] See Table 1 for diet composition.

Table 14. Analysis of Variance for Liver Parameters in Experiment 1

Source of variation	d.f.	Mean squares				
		Liver weight	Liver lipid wet basis	Total liver lipid	Liver as part of body weight	Final body weight
Treatment	3	571.50**	374.29**	233.78**	1.82**	.060
Grower diet	1	3.56	2.56	.09	.10	.010
Strain	2	415.29**	38.45	51.83	1.21**	.005
T x D	3	175.68*	57.06	55.92	.28	.083*
T x S	6	80.13	42.45	20.57	.09	.110*
D x S	2	78.85	55.96	21.94	.02	.050
T x D x S	6	109.45	46.79	34.31	.36	.038
Residual	48	49.31	33.21	18.87	.17	.023

* $P < .05$.
** $P < .01$.

can be noted due to layer diet treatment. Liver weight, expressed as absolute or as percent of body weight, was decreased by biotin ($P<.05$) and choline ($P<.01$) supplementation. The combination of both supplements decreased liver weight greater than either alone. Similar trends in liver lipid accumulation can be noted. Choline and biotin decreased liver lipid with choline having the most consistent and dramatic effect, reducing liver lipid by 50%. No effects on final body weights were observed.

The previous grower diet or strain of bird showed less effect on liver parameters. However, liver weight was significantly lower ($P<.01$) for the Hyline strain as compared to the Babcock strain.

The effects of choline on liver lipid conflict with some effects reported in previous studies. Some showed benefits, while others have found no effects. The data reported by Jensen et al. (1974b) are in close agreement with the results of this experiment, since choline supplementation decreased liver lipid by 16% or about one-half based on total liver lipid content.

The supplementation of biotin was less dramatic than choline on liver lipids in this experiment. However, biotin significantly reduced liver lipids and weight. This effect has not been reported in the literature. Chah et al. (1975) and Jensen et al. (1976b) reported no effects on liver lipids with biotin supplementation.

Experiment 2. Average production data and the analysis of variance for egg production, feed consumption, egg weight and feed efficiency are shown in Tables 15 and 16, respectively. The oats and

Table 15. Effect of Energy Source and Fat Level on Production Parameters--Experiment 2[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Feed per dozen eggs kg
Layer diet[c]				
Corn	69.7	96.2	62.5	1.63
Oats	59.2A	93.6	63.4	1.88a
Wheat	60.8A	96.7	59.2A	1.94A
Fat level				
2%	63.5	98.7	61.8	1.88
5%	63.0	92.2	61.5	1.75

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diet, Dunnett's t was used to test difference from corn diet.

[b] Values are average for 13, 28-day periods.

[c] See Table 3 for diet composition.

Table 16. Analysis of Variance for Production Parameters in Experiment 2

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Feed per dozen eggs
Replicates	4	356.30	75.82	40.76	.565
Fat level	1	25.33	4230.61	7.39	1.654
R x F	4	154.31	598.98	5.18	.368
Diet	2	4191.11**	357.74	640.54**	3.621**
R x D	8	235.24	268.19	12.92	.369
F x D	2	311.04	1454.04*	59.70*	.622
R x F x D	8	170.86	252.63	9.45	.190
Months	12	1254.47**	4746.54**	554.41**	3.929**
R x M	48	38.51	87.62	3.06	.115
F x M	12	34.16	52.89	3.76	.049
R x F x M	48	21.21	63.45	2.48	.039
D x M	24	147.05	236.76**	4.31*	.365**
R x D x M	96	28.99	78.15	2.61	.087
F x D x M	24	27.74	81.47	2.61	.106**
RFDM	96	25.66	56.24	2.20	.045

* $P < .05$.
** $P < .01$.

wheat diets did not support egg production at as high a rate as the corn control diet, probably the oats effect was due to its lower energy content. The wheat diet also suppressed egg size significantly ($P < .01$), probably due to a lower linoleic acid content. No significant effects could be noted due to the level of fat addition in the diet. Lowered feed consumption and improved feed efficiency for hens on the 5% added fat level approached the 5% level of significance. However, its interaction with diet probably prevented this significant effect.

Treatment data showing effects on final body weight and liver parameters are shown in Tables 17 and 18. The most striking difference was the lower liver weight and lipid levels in hens on the oats diets. This occurred even though final body weight was intermediate to that of the corn- and wheat-fed hens. No liver hemorrhages were noted in the oats-fed birds. These effects could be attributed to lowered energy consumption for the oats-fed hens.

A significantly ($P < .01$) higher level of hemorrhages was noted in the DeKalb strain. However, this was not the result of higher lipid levels as in the case of the layer diet treatment. The Hyline hens again showed less lipid accumulation than the other strains. This effect was not as significant as in Experiment 1. The level of dietary fat additions had no effect on liver parameters. Examination of individual liver data indicated that 30% of the livers had some degree of hemorrhage present (27 of 90). Average hepatic lipid for these livers was 16.5% (wet basis) while nonhemorrhaged livers averaged 8.7%. Liver lipid <u>per se</u> did not necessarily indicate FLHS, however, since

Table 17. Effect of Energy Source and Fat Level on Liver Parameters--Experiment 2[a,b]

Treatment	Liver weight	Liver score[c]	Total liver lipid	Liver lipid wet basis	Liver as part of body weight	Final body weight
	g		g	%	%	kg
Layer diet[d]						
Corn	44.4	1.8	6.07	12.7	2.36	1.89
Oats	36.9A	1.0A	2.07A	5.5A	2.10a	1.76a
Wheat	42.9	1.9	6.87	14.9	2.61a	1.66A
Strain						
Babcock B-300	40.9	1.3	5.29	11.6	2.34	1.74
DeKalb 231	42.4	2.1A	5.57	11.9	2.42	1.76
Hyline W-36	40.9	1.4	4.15	9.6	2.31	1.80
Fat level						
2%	41.4	1.5	4.76	10.3	2.36	1.77
5%	41.4	1.6	5.20	11.8	2.36	1.76

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diet and strain effects, Dunnett's t was used to test for difference from the corn diet or highest value, respectively.

[b] Hens were 76 weeks of age when sacrificed.

[c] 1 = 0 hemorrhages, 2 = 1 to 10 hemorrhages, 3 = 10 to 25 hemorrhages and 4 = greater than 25 hemorrhages.

[d] See Table 3 for diet composition.

Table 18. Analysis of Variance for Liver Parameters in Experiment 2

Source of variation	d.f.	Liver weight	Liver score	Total liver lipid	Liver lipid wet basis	Liver as part of body weight	Final body weight
Diet	2	474.03**	6.88**	195.25**	720.06**	1.890**	.385**
Fat level	1	.04	.18	5.23	50.58	.000	.003
Strain	2	22.53	5.41**	16.77	49.89	.093	.033
D x F	2	183.00	.42	31.06	45.65	.028	.233
S x D	4	143.37	1.25	50.18**	107.15**	.260	.042
S x F	2	19.38	1.15	11.73	26.29	.015	.013
S x D x F	4	57.85	1.78	.69	13.35	.107	.058
Residual	72	72.21	.72	10.53	21.39	.166	.051

* $P < .05$.
** $P < .01$.

several livers as low as 6 to 8% lipid had liver scores of 3 or 4, while others with as high as 28% lipid did not have any hemorrhages.

Jensen et al. (1976e) compared several grain sources for their effects on liver lipids. Their work agrees with these data in that oats as the primary carbohydrate source will decrease hepatic lipid by more than one-half. Their wheat-based diets decreased liver lipid by 25 to 40%, which does not agree with these data. Kim et al. (1976) found wheat diets to produce livers with hepatic lipids equal to that of corn diets.

The results of several researchers are in close agreement with those of this study on the effect of adding dietary fat to the layer diet. Price et al. (1957), McDaniel et al. (1959), Jensen et al. (1974b), Chah et al. (1975) and Ragland et al. (1970) showed no change in liver parameters measured from adding 0 to 10% animal fat.

Experiment 3. Average production data and the analysis of variance for egg production, feed consumption, egg weight and feed efficiency are shown in Tables 19 and 20, respectively. A small increase in egg production for birds raised on the oat groats diet is evident. The DeKalb strain of hens produced significantly more eggs than the Babcock or Hyline strain while being more efficient than the Babcock strain.

The greatest effects on production were observed due to the layer diet. The 12% protein diet could not support normal egg production, even though hens on this treatment consumed significantly more feed and produced significantly smaller eggs.

Table 19. Effect of Protein Level in Layer Diet, Grower Diet and Strain on Production Parameters--Experiment 3[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Feed per dozen eggs kg
Grower diet[c]				
1	65.5	99.2	63.1	1.82
2	65.3	98.5	62.8	1.80
3	65.3	98.9	62.7	1.81
4	66.5a	99.7	62.5	1.80
Strain				
Babcock B-300	63.4A	102.9	62.9	1.97
DeKalb 231	67.9	98.3A	62.6	1.72A
Hyline W-36	65.6A	96.1A	62.8	1.74A
Layer diet[d]				
16%	67.5A	98.2b	63.1A	1.74B
12%	63.7B	99.9a	62.4B	1.88A

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For grower diet and strain, Dunnett's t was used to test for difference from diet 1 and the highest value, respectively.

[b] Values are average of 13, 28-day periods.

[c] See Table 4 for diet composition.

[d] See Table 5 for diet composition.

Table 20. Analysis of Variance for Production Parameters in Experiment 3

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Feed per dozen eggs
Replicates	5	516.42	587.96	22.66	.275
Grower diet	3	151.15*	120.72	28.22	.063
R x D	15	37.89	161.02	30.79	.126
Strains	2	3198.60**	7405.68**	7.64	11.943**
R x S	10	107.55	130.53	13.71	.187
D x S	6	250.39	52.83	33.06	.277
R x D x S	30	130.91	107.19	21.80	.143
Treatments	1	6800.92**	1380.03*	255.88**	9.014**
R x T	5	181.40	175.81	4.73	.153
D x T	3	229.28	118.55	21.05	.311
R x D x T	15	170.61	125.56	27.79	.117
S x T	2	27.10	48.90	1.34	.079
R x S x T	10	230.03	227.26	25.87	.124
D x S x T	6	114.19	65.53	11.06	.092
RDST	30	141.37	112.72	11.53	.153
Months	12	5919.18**	14039.70**	2640.10**	14.415**
R x M	60	212.17	125.15	4.26	.168
D x M	36	25.05	19.84	1.35	.026
R x D x M	180	26.23	15.73	1.16	.031
S x M	24	206.92**	80.43**	13.01**	.350**
R x S x M	120	25.61	12.12	.90	.034
D x S x M	72	32.60	15.68	1.04	.029
RDSM	360	27.38	13.90	1.06	.032
T x M	12	590.06**	328.31**	6.27**	.252**
R x T x M	60	45.34	22.97	1.39	.045
D x T x M	36	35.10	17.63	1.12	.023
RDTM	180	28.55	16.09	1.00	.031
S x T x M	24	36.86	25.23*	2.54	.026
RSTM	120	21.63	13.82	1.27	.026
DSTM	72	21.43	9.66	.87	.026
RDSTM	360	25.25	11.64	1.10	.033

* $P < .05$.
** $P < .01$.

Treatment data showing effects on final body weight and liver parameters are shown in Tables 21 and 22. No effects due to grower diet were obtained on liver and body weight measurements. Protein level in the layer diet had no effects on the data given either. These results agree with other research reviewed in this paper (McDaniel et al., 1959; Owings et al., 1967; Quisenberry et al., 1967), which showed no effects on liver lipid with protein levels between 15 and 25%.

The Hyline strain showed the greatest ability to mobilize liver lipid. Their livers tended to be smaller whether based on absolute weight or as a percent of body weight. Liver score indicated significantly fewer hemorrhages present, while liver lipid levels were about 60% of that for the other strains. This strain effect is further verified in Table 23. Hens that died during the course of the experiment were taken to the South Dakota State University Diagnostic Laboratory for postmortem examination. No hens of the Hyline strain died of FLHS, while 6.5 and 16.7% of the mortality for the Babcock and DeKalb strains, respectively, were from FLHS.

Close examination of individual liver data indicated that 39% of the livers had some degree of hemorrhage present (28 of 72). Average hepatic lipid for these livers was 15.3% (wet basis), while livers scored as 1 averaged 8.8% fat. Although there were exceptions, the presence of hematomas was more indicative of actual hepatic lipid in this experiment. This agrees with the general observations of many researchers, including those of Wolford and Polin (1972c).

Table 21. Effect of Protein Level in Layer Diet, Grower Diet and Strain on Liver Parameters--Experiment 3[a,b]

Treatment	Liver weight	Liver score[c]	Liver lipid wet basis	Total liver lipid	Liver as part of body weight	Final body weight
	g		%	g	%	kg
Grower diet[d]						
1	42.3	1.5	11.5	5.26	2.17	1.82
2	42.3	1.7	13.0	5.64	2.26	1.87
3	39.3	1.6	9.2	4.05	2.26	1.77
4	42.1	1.9	11.6	5.30	2.26	1.84
Strain						
Babcock B-300	42.3	1.8	13.1	6.11	2.27	1.85
DeKalb 231	43.5	2.1	12.5	5.82	2.30	1.81
Hyline W-36	38.8	1.1A	8.4A	3.26A	2.14	1.82
Layer diet[e]						
9%	42.2	1.6	11.4	5.30	2.24	1.88
12%	42.8	1.7	11.3	4.83	2.24	1.77

[a] Values without a common subscript in each column and variable category are significantly different ($P < .01$). For grower diet and strain, Dunnett's t was used to test for difference from diet 1 and the highest value, respectively.

[b] Hens were 76 weeks of age at time of sacrifice.

[c] See Table 17.

[d] See Table 4 for diet composition.

[e] See Table 5 for diet composition.

Table 22. Analysis of Variance for Liver Parameters in Experiment 3

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Liver lipid wet basis	Total liver lipid	Liver as part of body weight	Final body weight
Grower diet	3	39.16	.56	45.15	8.75	.043	.034
Strain	2	141.35	6.13**	160.59*	59.09**	.180	.009
Treatment	1	36.13	.22	.26	4.06	.000	.230
D x S	6	43.27	.74	56.26	16.22	.240	.020
D x T	3	86.20	1.60	31.10	9.72	.396	.005
S x T	2	94.29	.82	122.34*	33.23	.372	.106
D x S x T	6	95.37	.73	47.64	13.57	.230	.035
Residual	48	85.01	.85	32.29	13.73	.250	.073

* $P < .05$.
** $P < .01$.

Table 23. Causes of Mortality by Strain in Experiment 3[a]

Disease	Babcock B-300	DeKalb 231	Hyline W-36
Leukosis	19.6	7.4	36.0
Cannibalism	30.4	53.7	8.3
Fatty liver-hemorrhagic syndrome	6.5	16.7	.0
Reproductive failure	15.2	13.0	16.7
Other	28.3	7.4	38.9

[a] Percent of mortality as determined by the South Dakota State University Diagnostic Laboratory.

Experiment 4. Treatment data showing effects on several production parameters are shown in Tables 24 and 25. Adding cellulose, oat hulls or wheat bran as a fiber source in isocaloric diets resulted in no differences in production parameters measured. The Regional Control SCWL hens showed significantly lower or less efficient production than the Shaver strain, an indication of the superiority of hybrid strains for egg production.

Treatment data showing the effects on final body weight and several liver parameters are shown in Tables 26 and 27. The isocaloric addition of fiber to the control diet did not decrease liver lipids. As evidenced by these data, the oat hull or bran of wheat is not the reason for the lipid suppressing effects of oats and wheat as reported elsewhere in this study and by other researchers (Jensen et al., 1976e; Kim et al., 1976).

The data in Table 26 indicate that commercial hybrid chickens have the ability to mobilize liver lipids more efficiently than the Regional Control straightbred hen. Liver weight and lipid measurements were significantly higher than those for the Shaver strain in all measurements taken. Body weight was also higher but not significantly so. The high level of fat in the livers of the Regional Control hens was in excess of that from birds on all other ad libitum experiments using commercial hybrid strains in these studies.

Close examination of individual liver data indicated that 29% of all livers had hematomas present. Hepatic lipid for these livers

Table 24. Effect of Strain and Layer Diet on Production Parameters--Experiment 4[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Feed per dozen eggs kg
Layer diet[c]				
Control	63.3	108.7	60.0	2.17
+ cellulose	65.2	104.9	59.7	2.00
+ oat hulls	62.9	100.8	58.8	2.03
+ wheat bran	61.9	101.0	59.7	2.01
Strain				
Shaver 288	69.2A	106.8a	61.9A	1.89b
Regional Control	57.5B	100.9b	57.2B	2.21a

[a] Average for nine, 28-day periods.
[b] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$).
[c] See Table 6 for diet composition.

Table 25. Analysis of Variance for Production Parameters in Experiment 4

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Feed per dozen eggs
Replicates	4	143.8	133.8	78.46	.121
Strain	1	12375.1**	3182.6*	1963.72**	8.836*
R x S	4	354.9	235.1	43.59	1.070
Treatments	3	174.0	1263.0	25.09	.574
R x T	12	430.5	665.6	14.69	.679
S x T	3	152.3	502.5	4.32	.061
R x S x T	12	365.6	466.9	18.13	.758
Months	8	5565.2**	5064.7**	657.41**	8.372**
R x M	32	109.1	81.1	1.69	.260
S x M	8	109.7**	107.2**	7.70**	.270
R x S x M	32	25.0	31.9	1.68	.140
T x M	24	37.1	71.8	2.23	.103
R x T x M	96	81.2	53.5	1.80	.217
S x T x M	24	56.2	69.9	1.70	.176
R x S x T x M	96	55.7	57.0	1.42	.203

* $P < .05$.
** $P < .01$.

Table 26. Effect of Strain and Layer Diet on Liver Parameters--Experiment 4[a,b]

Treatment	Liver weight	Liver score[c]	Total liver lipid	Liver lipid wet basis	Liver as part of body weight	Final body weight
	g		g	%	%	kg
Layer diet[d]						
Control	49.4	1.8	7.28	14.2	2.43	2.04
+ cellulose	47.2	1.7	6.29	12.5	2.54	1.89
+ oat hulls	49.1	1.4	7.35	13.0	2.44	2.01
+ wheat bran	46.0	1.7	5.37	10.4	2.36	1.95
Strain						
Shaver 288	44.5B	1.3B	5.09b	10.9b	2.32b	1.92
Regional Control	51.4A	2.0A	8.06a	14.2a	2.56a	2.02

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$).
[b] Hens were 64 weeks of age when sacrificed.
[c] See Table 17.
[d] See Table 6 for diet composition.

Table 27. Analysis of Variance for Liver Parameters in Experiment 4

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Total liver lipid	Liver lipid wet basis	Liver as part of body weight	Final body weight
Strain	1	952.2**	10.52**	177.3*	227.6*	1.19*	.19
Layer diet	3	53.7	.65	17.5	49.6	.11	.09
S x D	3	107.9	.68	38.3	46.3	.07	.04
Residual	72	139.0	1.12	27.4	39.1	.22	.09

* $P < .05$.
** $P < .01$.

averaged 16.1%, while those without hemorrhages averaged 11.1% on a wet basis. The number of hemorrhages was not correlated to the level of fat, however. Livers scored as 2, 3 or 4 averaged 18.6, 13.9 and 14.4% fat, respectively.

Force-Fed Experiments

Experiment 5. Treatment data showing effects of several production parameters are shown in Tables 28 and 29. Force-feeding a control 14% protein diet supplemented with choline and/or biotin had no effect on the parameters measured. The Hyline strain tended to consume less feed, resulting in lower egg production, significantly smaller egg size and less body weight gain. The Babcock strain also had a significantly smaller egg size than the Shaver strain.

Birds raised on the 10% protein grower diet laid significantly smaller eggs and had a smaller body size than those raised on the 12% protein grower diet.

Treatment data showing effects on final body weight and several liver parameters are shown in Tables 30 and 31. The addition of choline and/or biotin had no effects on liver lipid accumulation under these force-feeding conditions as compared to ad libitum feeding in Experiment 1. No effect due to previous grower diet could be noted.

The Hyline strain had significantly more liver hemorrhages in this experiment, but lipid analysis showed the livers to be significantly lower in accumulated fat by other measurements.

Table 28. Effect of Strain and Grower and Layer Diets on Production Parameters--Experiment 5[a,b]

Treatment	Hen-day production	Hen-day feed consumed	Egg weight	Final body weight
	%	g	g	kg
Layer diet[c]				
Control	72.5	112	60.6	2.01
+ choline	79.3	116	59.8	2.02
+ biotin	80.7	114	59.9	1.93
+ choline and biotin	81.3	115	58.2	1.94
Strain				
Babcock B-300	80.8	117	59.4A	2.00
Shaver 288	79.2	116	61.5	1.98
Hyline W-36	75.5	111	58.0A	1.95
Grower diet[d]				
12%	78.5	116	60.8A	2.06a
10%	78.4	112	58.4B	1.90b

[a] Values without a common subscript within each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diet and strain effect, Dunnett's t was used to test difference from control or highest value, respectively.

[b] Average for 5 weeks of force-feeding.

[c] See Table 2 for diet composition.

[d] See Table 1 for diet composition.

Table 29. Analysis of Variance for Production Parameters in Experiment 5

Source of variation	d.f.	Mean squares			
		Hen-day production	Hen-day feed consumed	Egg weight	Final body weight
Treatment	3	292.65	52.50	18.59	.039
Grower diet	1	.02	300.13	103.68**	.439*
Strain	2	177.59	255.18	71.67**	.013
T x D	3	152.12	68.16	4.48	.130
T x S	6	80.38	66.27	14.47	.043
D x S	2	5.61	38.38	12.58	.076
T x D x S	6	127.38	49.25	5.86	.025
Residual	48	217.93	108.17	12.45	.087

* $P \leq .05$.
** $P < .01$.

Table 30. Effect of Strain and Grower and Layer Diets on Several Liver Parameters--Experiment 5[a,b]

Treatment	Liver weight	Liver score[c]	Total liver lipid	Liver lipid		Liver as part of body weight
				Wet basis	Dry basis	
	g		g	%	%	%
Layer diet[d]						
Control	62.9	1.72	14.6	22.2	47.9	3.15
+ choline	66.1	2.17	13.4	17.6	42.5	3.27
+ biotin	66.2	2.33	15.1	22.0	48.2	3.43
+ choline and biotin	65.8	1.78	12.5	18.4	43.5	3.38
Strain						
Babcock B-300	68.8	2.08	16.8	23.5	50.5	3.41
Shaver 288	63.3	1.50a	12.6a	19.5a	45.4	3.23
Hyline W-36	63.7	2.42	12.2a	17.1A	40.7a	3.28
Grower diet[e]						
12%	67.7	1.94	15.1	21.7	47.9	3.32
10%	62.8	2.06	12.7	18.4	43.2	3.30

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer and strain effect, Dunnett's t was used to test for differences from control or the highest value, respectively.

[b] Hens were 32 weeks of age when sacrificed after 21 days of force-feeding.

[c] See Table 17.

[d] See Table 2 for diet composition.

[e] See Table 1 for diet composition.

Table 31. Analysis of Variance for Several Liver Parameters in Experiment 5

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Total liver lipid	Liver lipid: Wet basis	Liver lipid: Dry basis	Liver as part of body weight
Treatment	3	45.1	1.59	25.39	103.78	157.8	.280
Grower diet	1	430.2	.22	105.12	190.13	400.9	.009
Strain	2	232.2	5.17*	157.14*	249.62**	570.4*	.220
T x D	3	53.6	2.26	74.63	64.44	173.5	.320
T x S	6	86.5	.76	25.57	35.98	77.2	.176
D x S	2	110.1	1.39	172.84*	24.01	103.7	.396
T x D x S	6	141.8	1.20	47.56	42.20	116.1	.350
Residual	48	124.0	1.24	47.79	48.58	116.3	.310

* $P < .05$.
** $P < .01$.

Examination of individual liver data indicated that 54% (39 of 72) of the force-fed birds had liver hematomas at the time of sacrifice. However, there was essentially no difference in liver fat between the two groups, with livers scored as 1 averaging 19.5% lipid and those with hemorrhages averaging 20.6% fat. Since all hens in this experiment were force-fed, the indication is that some hens are more genetically susceptible to the liver hemorrhaging when the level of liver fat reaches a high level. Ivy and Nesheim (1973) have indicated that genetic susceptibility to hemorrhage may be a factor.

Experiment 6. This experiment was conducted to determine what levels of force-feeding are required to get a high incidence of FLHS. Production data from force-feeding corn or wheat diets at three levels of consumption are shown in Tables 32 and 33. Hens on the wheat diet did not perform as well as those on the corn diet, possibly due to the lower energy content of the wheat diet. Egg production, egg weight and body weight gain were less on the wheat diet, giving results similar to those obtained in Experiment 2.

Increasing the level of feed consumption did not affect egg production during the 21-day experiment. Body weight gain and egg weight, however, increased as energy consumption increased. The analysis of variance showed egg weights between feeding regimes to be significant. However, when using Dunnett's t test for differences, a $S_{\bar{D}}$ value of 4.2 g is needed for significance at $P < .05$.

Treatment data showing effects on several liver parameters are shown in Tables 34 and 35. In this experiment, the wheat diet

Table 32. Effect of Energy Source and Level of Force-Feeding on Production Parameters--Experiment 6[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Body weight gain kg
Layer diet[c]				
Corn	76.5	101	56.5a	.240A
Wheat	73.7	103	53.8b	.170B
Feeding regime				
Normal	77.5	86	53.5	.074
120%	73.4	102A	54.6	.250A
140%	74.5	118A	57.4	.290A

[a] Values without a common subscript within each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For effect of feeding regime, Dunnett's t was used to test for differences from the normal.

[b] DeKalb hens used in the 3-week force-feeding experiment were 33 weeks of age at time of sacrifice.

[c] See Table 7 for diet composition.

Table 33. Analysis of Variance for Production Parameters in Experiment 6

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
Diet	1	58.18	40.84	54.50*	.0520**
Method	2	45.04	2640.64**	40.66*	.1400**
D x M	2	3.90	2.23	32.14	.0125*
Residual	24	166.28	49.90	11.31	.0028

* $P < .05$.
** $P < .01$.

Table 34. Effect of Energy Source and Level of Force-Feeding on Liver Parameters--Experiment 6[a,b]

Treatment	Liver weight g	Liver score[c]	Liver lipid wet basis %	Total liver lipid g	Liver as part of body weight %
Layer diet[d]					
Corn	57.8A	2.2	22.2	14.9A	3.11a
Wheat	43.9B	1.8	18.1	8.5B	2.65b
Feeding regime					
Normal	34.8	1.1	12.5	4.7	2.25
120%	52.1A	2.1a	22.0A	12.3A	2.97A
140%	65.7A	2.8A	26.0A	18.1A	3.43A

[a] Values without a common subscript within each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For effect of feeding regime, Dunnett's t was used to test for difference from the normal.
[b] See Table 32.
[c] See Table 17.
[d] See Table 7 for diet composition.

Table 35. Analysis of Variance for Liver Parameters in Experiment 6

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Liver lipid wet basis	Total liver lipid	Liver as part of body weight
Diet	1	1454.9**	1.26	125.9	307.1**	1.61*
Method	2	2400.2**	7.18**	486.1**	449.4**	3.57**
D x M	2	391.7	2.04	218.4**	127.8	1.26*
Residual	24	129.3	.92	35.3	34.3	.34

* $P < .05$.
** $P < .01$.

significantly lowered liver weight whether based on absolute or on percent of body weight. Total liver lipid was significantly reduced by about 40%, which is in agreement with the Jensen et al. (1976e) work.

Increasing feed consumption produced a concomitant increase in all liver parameters. The dose-response relationship between feed intake and liver hemorrhage score or liver lipid content as established by Wolford and Polin (1974) was verified. Close examination of individual liver data indicated that increasing levels of feeding produced hemorrhages on 10, 60 and 70% of the birds, respectively. Livers with no hemorrhages averaged 15.8% fat, while livers scored 2, 3 or 4 averaged 17.9, 26.8 and 29.5% fat, respectively. Data from this experiment indicate that a force-feeding level of 125 to 130% of normal intake will produce FLHS in 60 to 70% of the hens, which should be an adequate level of incidence when studying this syndrome.

Experiment 7. In several of the reports previously discussed, high levels of fat were utilized in an attempt to cause increased incidence of FLHS. Levels as high as 10% fat, however, did not cause a high incidence of the disease. This experiment was conducted to determine if isocaloric diets with 2, 5 or 8% added fat would affect liver lipid accumulation.

Treatment data showing effects of layer diet and feeding regime on several production parameters are shown in Tables 36 and 37. Fat level in the layer diet showed no significant effects on the parameters measured. Egg weight appeared to be depressed with increasing levels

Table 36. Effect of Dietary Fat Level and Feeding Regime on Production Parameters--Experiment 7[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Body weight gain kg
Layer diet[c]				
2% fat	84.3	124	63.4	.260
5% fat	77.6	123	60.9	.260
8% fat	86.7	123	59.4	.210
Feeding regime				
Ad libitum	90.5A	115B	58.9B	.134B
Force-fed	75.3B	131A	63.6A	.349A

[a] Values without a common subscript within each column and variable category are significantly different ($P < .01$).

[b] Shaver hens were 33 weeks of age after the 3-week force-feeding experiment and time of sacrifice.

[c] See Table 8 for diet composition.

Table 37. Analysis of Variance for Production Parameters in Experiment 7

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
Diet	2	220.1	2.64	41.02	44.4
Method	1	1738.9**	2050.13**	171.85**	697.7**
D x M	2	397.2	126.24	17.00	29.3
Residual	24	209.6	72.75	23.18	37.8

** $P < .01$.

of added dietary fat, but variability within treatments prevented the depression from being significant.

The force-feeding regime caused highly significant effects on all parameters measured. The reduced egg production and increased body weight were typical of FLHS. The ad libitum fed birds consumed an unusually large amount of feed considering the energy level of these diets. This resulted in a force-feeding level of intake of only 114% of that of the ad libitum fed birds. However, even the relatively smaller increase in energy consumption by the force-fed birds resulted in an increase in egg size.

Treatment data showing effects on several liver parameters are shown in Tables 38 and 39. The level of dietary fat resulted in no significant effect on the incidence of FLHS. However, there was a tendency for a decrease in liver parameters as dietary fat level increased. The force-feeding regime again increased the incidence of FLHS. Examination of individual liver data indicated that no hemorrhages occurred in the ad libitum fed birds, while 46% (7 of 15) of the force-fed birds had liver hematomas. Livers that were scored as 1 averaged 15.2% lipid, while those scored as 2, 3 or 4 averaged 20.5, 25.8 and 30.8% fat, respectively.

Experiment 8. This experiment repeated the treatments used in Experiment 7. However, in order to better control feed consumption, the self-fed birds were restricted to about 100 g of feed per day, while the force-fed birds were given 125 g per day with no additional

Table 38. Effect of Dietary Fat Level and Feeding Regime on Liver Parameters--Experiment 7[a,b]

Treatment	Liver weight %	Liver score[c]	Total liver lipid g	Liver lipid wet basis %	Liver as part of body weight %
Layer diet[d]					
2% fat	56.1	1.8	10.6	16.8	2.86
5% fat	58.4	1.3	12.3	19.9	2.94
8% fat	50.9	1.2	8.9	15.9	2.62
Feeding regime					
Ad libitum	44.9B	1.0B	6.1B	12.8B	2.41B
Force-fed	65.4A	1.9A	15.1A	22.3A	3.21A

[a] Values without a common subscript within each column and variable category are significantly different ($P < .01$).
[b] See Table 36.
[c] See Table 17.
[d] See Table 8 for diet composition.

Table 39. Analysis of Variance for Liver Parameters in Experiment 7

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Total liver lipid	Liver lipid wet basis	Liver as part of body weight
Diet	2	147.7	1.05	28.1	44.4	.29
Method	1	3162.2**	5.67**	615.7**	679.7	4.72**
D x M	2	85.6	1.02	15.0	29.3	.11
Residual	24	95.0	.48	31.3	37.8	.28

** $P < .01$.

ad libitum feeding allowed. Data showing the effects of treatment on several production parameters are shown in Tables 40 and 41. Again, no effects due to added dietary fat were evident. The force-fed birds showed signs typical of FLHS with a significant decrease in production and a significant increase in weight ($P < .01$). As in previous experiments, the increase in energy consumption significantly increased ($P < .05$) egg weight.

Treatment data showing effects of layer diet and feeding regime on several liver parameters are shown in Tables 42 and 43. The level of dietary fat had no significant effects on liver parameters. However, there again was the tendency for liver lipids to decrease with increases in dietary fat level. Force-feeding again increased the level of FLHS. However, some incidence of the disease occurred in the self-fed birds which was probably the result of the older age of the birds used in this experiment as compared to those in Experiment 7. Examination of individual liver data indicated that 40% of the self-fed birds had liver hematomas as compared to 73% of the force-fed birds. Livers scored as 1 averaged 16.9% lipid, while those with hemorrhages averaged 19.0%, indicating that liver lipid per se was not the controlling factor for hemorrhage tendency.

Experiment 9. Treatment data showing effects of layer diet and feeding regime on several production parameters are shown in Tables 44 and 45. Layer diet treatments had no effects on production parameters except for a small but significant increase in body weight on the

Table 40. Effect of Dietary Fat Level and Feeding Regime on Production Parameters--Experiment 8[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Body weight gain kg
Layer diet[c]				
2% fat	67	115	64.3	.152
5% fat	65	113	66.0	.201
8% fat	62	112	65.1	.108
Feeding regime				
Ad libitum[d]	78A	102B	63.9b	.036B
Force-fed	51B	125A	66.4a	.270A

[a] Values without a common subscript within each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$).

[b] Shaver 288 hens were 51 weeks of age after the 3-week force-feeding experiment and at the time of sacrifice.

[c] See Table 8 for diet composition.

[d] Restricted to 100 g per day.

Table 41. Analysis of Variance for Production Parameters in Experiment 8

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
Diet	2	69.1	13.44	7.76	.020
Method	1	5216.7**	3990.54**	47.63*	.414**
D x M	2	327.3	20.15	30.61	.013
Residual	24	354.3	15.07	11.09	.015

* $P < .05$.
** $P < .01$.

Table 42. Effect of Dietary Fat Level and Feeding Regime on Liver Parameters--Experiment 8[a,b]

Treatment	Liver weight	Liver score[c]	Total liver lipid	Liver lipid wet basis	Liver as part of body weight
	g		g	%	kg
Layer diet[d]					
2% fat	55.0	2.0	11.4	18.3	2.81
5% fat	51.0	1.8	11.0	19.1	2.66
8% fat	46.2	2.1	6.6	13.9	2.45
Feeding regime					
Ad libitum[e]	42.8B	1.6	5.5B	12.7B	2.48
Force-fed	58.9A	2.3	13.9A	21.5A	2.80

[a] Values without a common subscript within each column and variable category are significantly different ($P < .01$).
[b] See Table 40.
[c] See Table 17.
[d] See Table 8 for diet composition.
[e] Restricted to 100 g per day.

Table 43. Analysis of Variance for Liver Parameters in Experiment 8

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Total liver lipid	Liver lipid wet basis	Liver as part of body weight
Diet	2	195.7	.24	71.50	80.1	.34
Method	1	1952.1**	4.04	532.57**	592.3**	.75
D x M	2	261.7	.43	125.15	216.0**	.54
Residual	24	194.2	1.15	48.57	33.6	8.61

** $P < .01$.

Table 44. Effect of Choline and/or Methionine Supplementation in Force-Fed Diets on Production Parameters--Experiment 9[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Body weight gain kg
Layer diet[c]				
Control	66	138	67.5	.071
+ choline	67	134	68.7	.098
+ methionine	75	140	63.3	.129a
+ choline and methionine	75	130	67.4	.109
Feeding regime				
Ad libitum	74	122B	65.7	.013B
Force-fed	67	149A	67.8	.189A

[a] Values without a common subscript within each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diet, Dunnett's t was used to test for difference from control.

[b] Shaver 288 hens were used for this 3-week force-feeding experiment. They were 69 weeks of age at time of sacrifice.

[c] See Table 9 for diet composition.

Table 45. Analysis of Variance for Production Parameters in Experiment 9

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
Diet	3	343.0	259.8	76.93	.116**
Method	1	695.1	10505.2**	61.95	.440**
D x M	3	383.0	592.1*	150.26	.002
Residual	48	661.1	205.5	112.35	.004

* $P < .05$.
** $P < .01$.

methionine added diet. Choline supplementation did not improve egg production for the 3-week experimental period as in previous experiments. The force-fed hens again tended to show production signs of FLHS as a result of the increase in energy consumption.

Treatment data showing effects of layer diet and feeding regime on liver parameters are shown in Tables 46 and 47. Choline addition decreased total liver lipid in the ad libitum fed birds but had the opposite effect in the force-fed birds. This resulted in a significant diet-method interaction and, therefore, little overall effect on liver parameters due to choline supplementation. Additional methionine supplementation resulted in increases in several liver parameters measured.

The force-feeding technique was again successful in producing livers typical of FLHS with total lipid accumulation averaging four times that of the ad libitum fed birds. Examination of individual liver data revealed that 30 out of the 56 hens had liver hematomas (8 in the ad libitum and 22 in the force-fed). Livers with no hemorrhages averaged 15.8% lipid, while those scored as 2, 3 or 4 averaged 23.2, 24.3 and 33.4% fat, respectively. In this experiment, liver hemorrhages appeared to be a result of increased lipid content.

Experiment 10. Treatment data showing effects of layer diet and feeding regime on production parameters are shown in Tables 48 and 49. Egg production decreased to one-half with the oats diets fed ad libitum due to the drastic dietary change and reduced feed intake. The change

Table 46. Effect of Choline and/or Methionine Supplementation in Force-Fed Diets on Liver Parameters--Experiment 9[a,b]

Treatment	Liver weight g	Liver score[c]	Liver lipid wet basis %	Total liver lipid g	Liver as part of body weight %
Layer diet[d]					
Control	58.6	1.5	21.2	14.2	2.75
+ choline	64.6	2.4a	20.4	15.8	3.12
+ methionine	78.5	2.6A	26.5	25.1a	3.75a
+ choline and methionine	62.3	1.9	21.3	15.4	2.93
Feeding regime					
Ad libitum	46.4B	1.4B	13.9B	7.3B	2.30B
Force-fed	85.8A	2.8A	30.8A	28.0A	3.97A

[a] Values without a common subscript within each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diets, Dunnett's t was used to test for difference from control.
[b] See Table 44.
[c] See Table 17.
[d] See Table 9 for diet composition.

Table 47. Analysis of Variance for Liver Parameters in Experiment 9

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Liver lipid wet basis	Total liver lipid	Liver as part of body weight
Diet	3	1050.1	11.79**	109.8	351.6*	2.63*
Method	1	21685.8**	48.89**	4001.9**	5986.4**	38.93**
D x M	3	1065.8	.87	102.2	304.1*	2.74*
Residual	48	451.8	.97	53.0	107.5	.87

* $P < .05$.
** $P < .01$.

Table 48. Effect of Energy Source, Choline and Feeding Regime on Production Parameters--Experiment 10[a,b]

Treatment	Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
	%	g	g	kg
Layer diet[c]				
Corn-soy	73.2	129	67.1	.128
Oats-soy	41.3A	112	66.7	.108
Corn-soy + choline	68.1	125	65.5	.192
Oats-soy + choline	52.8a	132	66.2	.062
Feeding regime				
Ad libitum	57.1	95B	67.6	.004B
Force-fed	60.6	154A	65.1	.240A

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diets, Dunnett's t was used to test for differences from control (corn-soy).

[b] Shaver 288 hens were used in this 3-week force-feeding experiment. They were 76 weeks of age at the time of sacrifice.

[c] See Table 10 for diet composition.

Table 49. Analysis of Variance for Production Parameters in Experiment 10

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
Diet	3	2120.3**	779.9*	5.29	.0291**
Method	1	122.5	35343.0**	64.26	.5593**
D x M	3	1074.4	218.4	52.00	.0351**
Residual	32	402.0	228.0	18.48	.0040

* $P < .05$.
** $P < .01$.

did not occur in the force-fed oats diets, apparently because the hens were forced to consume enough calories to maintain production. This resulted in a significant diet-method interaction at $P < .06$. On a total diet basis, the oats diets were significantly lower in egg production, while force-feeding had no effect on production. The analysis of variance showed layer diets to have a significant effect on feed consumption and body weight gain. However, Dunnett's t test did not show a specific difference. The force-feeding technique had significant effects on feed consumption and body weight but did not affect egg production and egg weight as in previous experiments.

Treatment data showing effects of layer diet and feeding regime on liver parameters are shown in Tables 50 and 51. A significant interaction between layer diet and feeding method occurred for all liver parameters measured. This was due primarily to the low feed consumption (88.3 g per day) for the oats ad libitum fed birds. This low energy consumption led to low liver lipid values (.65 g per liver) as compared to the lipid values of the oats force-fed birds (7.71 g per liver).

Highly significant differences occurred between layer diets and feeding regime for all parameters even though interactions were evident. The most drastic layer diet effect occurred between the corn and oats diets. None of the hens on the oats diets, regardless of feeding regime, showed signs of FLHS. Total liver lipids for hens on oats were less than half of that of hens on corn diets. Some factor

Table 50. Effect of Energy Source, Choline and Feeding Regime on Liver Parameters--Experiment 10[a,b]

Treatment	Liver weight	Liver score[c]	Liver lipid wet basis	Total liver lipid	Liver as part of body weight
	g		%	g	%
Layer diet[d]					
Corn-soy	64.6	2.1	21.4	18.5	3.09
Oats-soy	39.1A	1.0A	6.5A	3.2A	1.98A
Corn-soy + choline	57.8	1.9	15.2a	11.9a	2.87a
Oats-soy + choline	47.1A	1.0A	8.5A	5.1A	2.28A
Feeding regime					
Ad libitum	31.7B	1.1B	4.0B	1.4B	1.86B
Force-fed	72.7A	2.0A	21.8A	18.0A	3.26A

[a] Values without a common subscript in each column and variable category are significantly different (capital letters = $P < .01$ and small letters = $P < .05$). For layer diets, Dunnett's t was used to test for differences from control (corn-soy).
[b] See Table 48.
[c] See Table 17.
[d] See Table 10 for diet composition.

Table 51. Analysis of Variance for Liver Parameters in Experiment 10

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Liver lipid wet basis	Total liver lipid	Liver as part of body weight
Diet	3	1275.8**	3.40**	461.5**	480.8**	2.64**
Method	1	16810.0**	8.10**	3159.5**	2748.1**	19.73**
D x M	3	698.0**	2.70**	175.7*	341.4**	1.58**
Residual	32	102.0	.49	43.3	49.0	.22

* $P < .05$.
** $P < .01$.

in oats apparently gives protection against liver lipid accumulation, even when feed consumption is extremely high (156 g per day for the force-fed oats diets). Choline supplementation reduced liver lipids in hens fed the corn diets but not in hens fed the oats diets. However, for both feeding regimes feed consumption averaged 20 g more per day for the choline supplemented oat diets.

The force-feeding technique increased total liver lipid by greater than tenfold while doubling liver size, whether based on absolute weight or percent of body weight. Close examination of individual liver data indicated that nine of the 40 hens on the experiment had liver hematomas, all of which were on the corn diets. Livers with no hemorrhages averaged 7.8% fat, while the nine livers with hemorrhages averaged 30.4% fat, giving a clear indication of the relationship of liver lipid level to liver hemorrhage.

Experiment 11. Treatment data showing effects of layer diet and feeding regime on production parameters are shown in Tables 52 and 53. Type of fiber addition in these isocaloric diets had no effects on any production parameters. The force-feeding technique again produced similar production effects that are typical of hens with FLHS. However, again egg production and egg weight were not significantly altered in this experiment.

Treatment data showing effects of layer diet and feeding regime on liver parameters are shown in Tables 54 and 55. The isocaloric addition of fiber to the control diet did not decrease liver lipids.

Table 52. Effect of Layer Diet and Feeding Regime on Production Parameters--Experiment 11[a,b]

Treatment	Hen-day production %	Hen-day feed consumed g	Egg weight g	Body weight gain kg
Layer diet[c]				
Control	51.9	116.5	61.4	.095
+ cellulose	59.0	127.5	61.9	.091
+ oat hulls	59.7	125.0	57.5	.122
+ wheat bran	45.7	121.3	61.1	.099
Feeding regime				
Ad libitum	58.1	103.8B	59.2	.014B
Force-fed	50.1	141.4A	61.8	.090A

[a] Values without a common subscript within each column and variable category are significantly different ($P < .01$).

[b] Regional Control SCWL hens were used in the 3-week force-feeding experiment. They were 68 weeks of age at the time of sacrifice.

[c] See Table 6 for diet composition.

Table 53. Analysis of Variance for Production Parameters in Experiment 11

Source of variation	d.f.	Mean squares: Hen-day production	Hen-day feed consumed	Egg weight	Body weight gain
Diet	3	438.0	228.9	41.25	.0020
Method	1	640.0	14175.2**	65.28	.3080**
D x M	3	815.5	110.9	52.63	.0010
Residual	32	482.5	96.8	24.19	.0035

** $P < .01$.

Table 54. Effect of Layer Diet and Feeding Regime on Liver Parameters--Experiment 11[a,b]

Treatment	Liver weight g	Liver score[c]	Total liver lipid g	Liver lipid wet basis %	Liver as part of body weight %
Layer diet[d]					
Control	72.6	2.6	23.1	26.8	3.36
+ cellulose	67.7	2.7	20.4	26.1	3.24
+ oat hulls	76.1	2.5	22.9	26.2	3.71
+ wheat bran	70.6	2.1	21.3	24.1	3.29
Feeding regime					
Ad libitum	46.0B	1.8	8.6B	17.2B	2.34B
Force-fed	97.6A	3.2	35.2A	34.4A	4.46A

[a] Values without a common subscript within each column and variable category are significantly different ($P < .01$).
[b] See Table 52.
[c] See Table 17.
[d] See Table 6 for diet composition.

Table 55. Analysis of Variance for Liver Parameters in Experiment 11

Source of variation	d.f.	Mean squares: Liver weight	Liver score	Total liver lipid	Liver lipid wet basis	Liver as part of body weight
Diet	3	124.6	.69	17.07	13.91	.46
Method	1	26625.6**	18.22	7081.98**	2960.46**	45.24**
D x M	3	236.7	2.96	35.17	7.80	.79
Residual	32	652.1	5.53	174.99	84.77	1.10

** $P < .01$.

This verifies the results obtained in Experiment 4, that the oat hull or bran of wheat is not the reason for the lipid suppressing effects of oats and wheat as reported elsewhere in this study and by other researchers (Jensen et al., 1976e; Kim et al., 1976).

The force-feeding technique again produced liver symptoms typical of FLHS, with most parameters showing a twofold increase over liver conditions of the ad libitum fed hens.

Close examination of individual data indicated a high level of FLHS in these Regional Control straightbred hens. Hemorrhages were evident on 50% of the livers from ad libitum fed hens and 90% of the livers from the force-fed birds. The 12 livers without hemorrhages averaged 15.0% fat, while those with hemorrhages averaged 30.4% fat.

Histological Observations

Attempts were made during the course of this study to compare individual livers for histological differences. Figure 1 shows two livers from hens that were in good egg production (83 and 74%, respectively) during the second force-feeding experiment (Experiment 6). Liver number 107 (considered a "normal liver") was removed from a hen on the wheat ad libitum treatment. It weighed 29 g and contained 7.3 g of lipid. Liver number 106 was removed from a hen on the 140% force-feeding corn treatment. It weighed 104 g and contained 38.6 g of lipid. This was considered a severe case of FLHS.

Figure 2 shows typical livers from the force-fed hens in Experiment 10 that had consumed about 150 g of feed per day. Hen

number 16 was in good production (80%), while number 15 had been out of production for ten days. The liver from the oats-fed hen was analyzed to contain only 2.8 g of lipid, while the liver from the corn-fed hen contained 13.7 g of lipid. The deep red color of the liver from number 16 was typical of all livers examined from birds fed diets containing oats in this study.

Figure 3 shows a close-up view of the necrotic condition of liver number 106 in Figure 1.

Figures 4 through 7 project a series of paraffin-mounted liver sections stained with hematoxylin and eosin that were analyzed and shown to contain 41.0, 31.0, 18.8 and 6.6% lipid, respectively (wet basis). Note the crowding of the nuclei and the disarrangement of the hepatic cells in Figure 4 in contrast to the cord-like arrangement of the cells and the pronounced sinusoid spaces for the liver section in Figure 7.

Figures 8 through 11 show a series of frozen liver sections stained with hematoxylin and eosin that were analyzed and shown to contain 47.0, 28.0, 13.5 and 2.3% lipid, respectively (wet basis). Figures 12 through 15 show sections from the same livers that were stained with oil red O to clearly show the infiltration of lipid. Figures 10 and 14 show normal livers to have fat droplets that are intracellular. FLHS inflicted hens (Figures 8 and 12) show an abundance of intracellular fat and extracellular lipid as well. Figures 11 and 15 are sections of liver from a hen that had been on an oats <u>ad libitum</u> treatment and had been out of egg production for

Fig. 1. Comparison of a "normal liver" and one from a hen with severe FLHS from the second force-feeding experiment. Number 107 was removed from a hen on the wheat ad libitum treatment, while number 106 was removed from a hen on the 140% force-feeding treatment. The first liver weighed 29 g and contained 7.3 g of lipid, while the latter liver weighed 104 g and contained 38.6 g.

Fig. 2. Typical livers from hens forced to consume about 150 g per day. Liver number 16 contained 2.8 g of lipid, while number 15 contained 13.7 g.

Fig. 3. A close-up view of the necrotic condition of liver 106 in Figure 1.

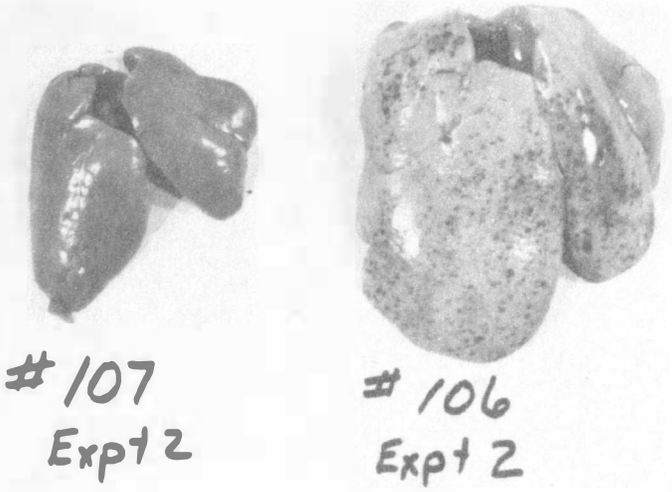


FIG. 1

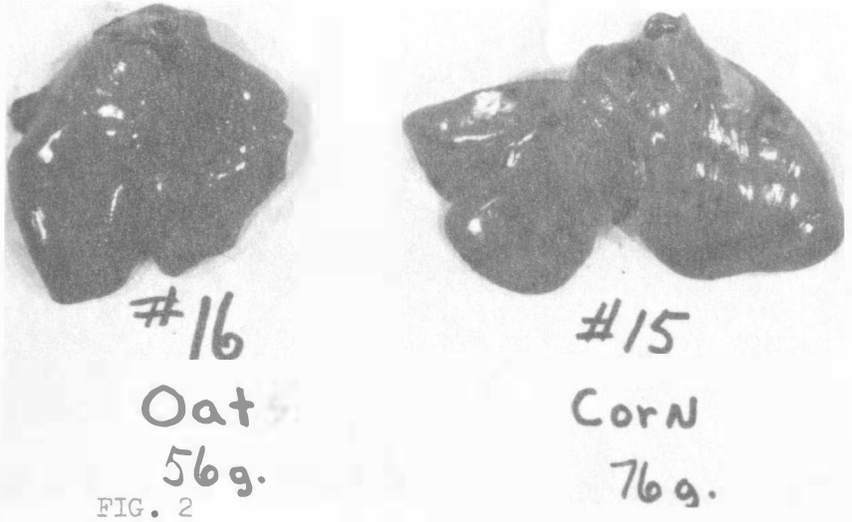


FIG. 2

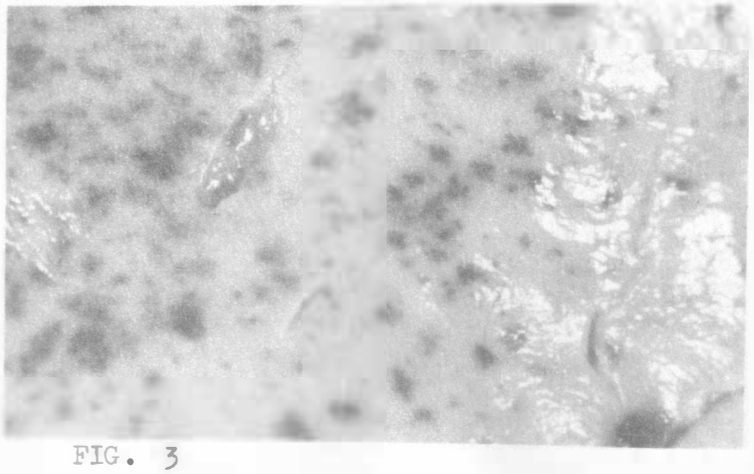


FIG. 3

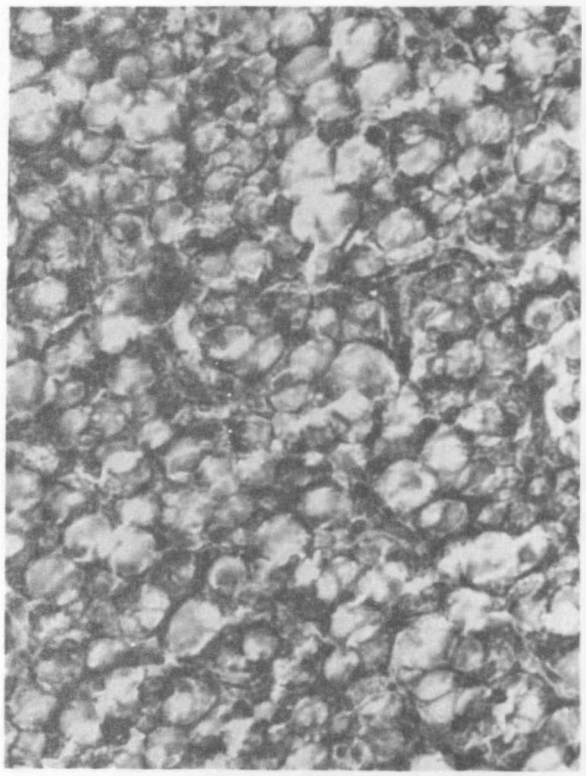


FIG. 4

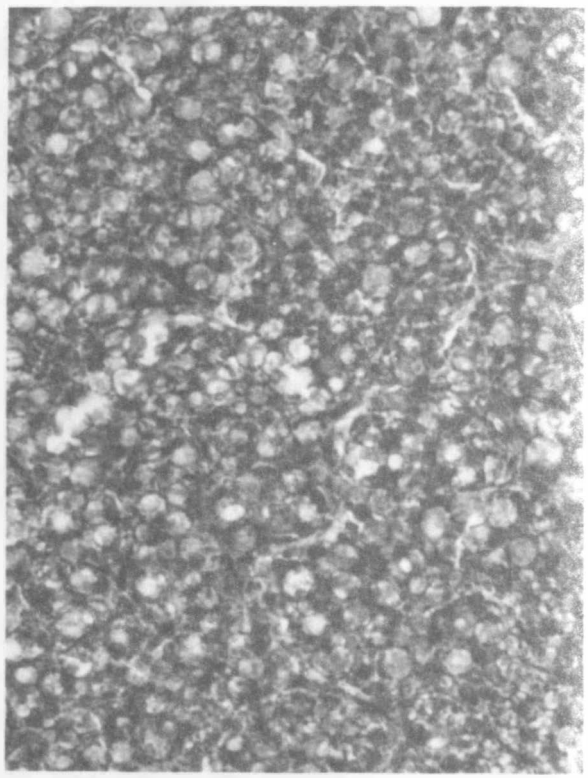


FIG. 5

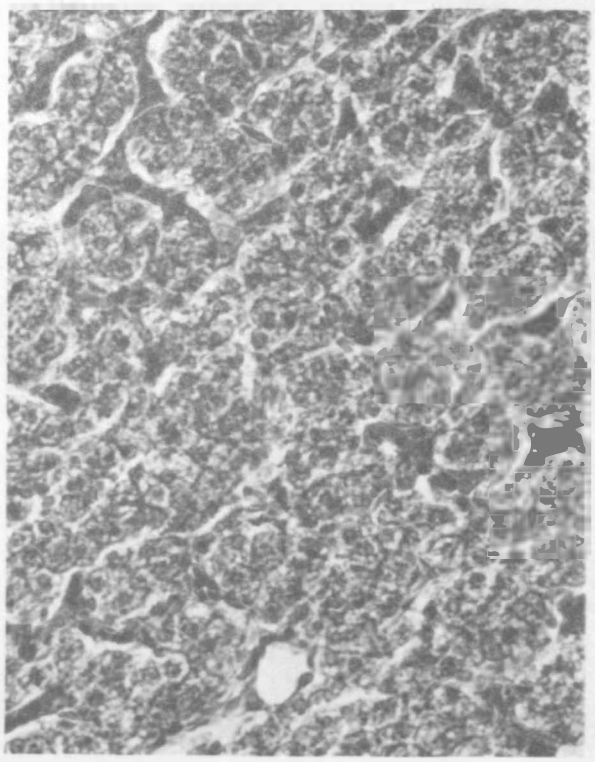


FIG. 6

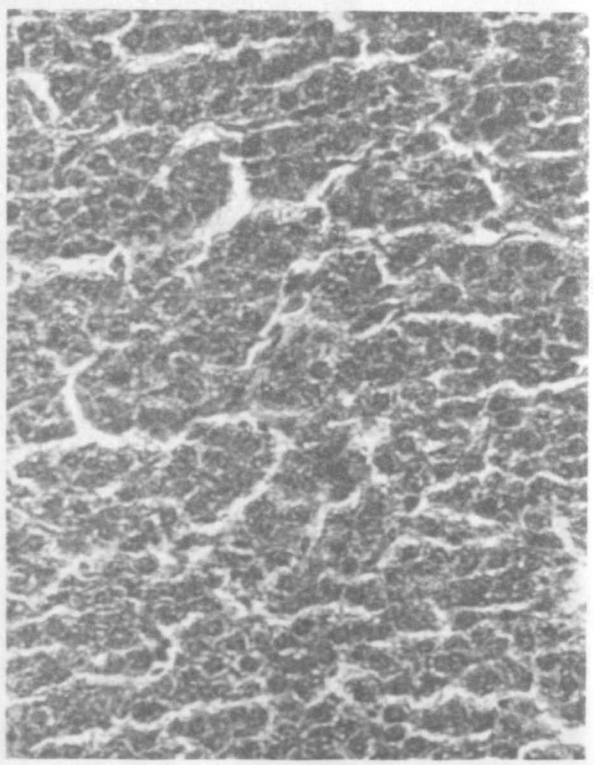


FIG. 7

Fig. 4-7. Photomicrographs of hen livers mounted in paraffin and stained with hematoxylin and eosin. The livers were analyzed and shown to contain 41.0, 31.0, 18.8 and 6.6% lipid, respectively (wet basis). Note the crowding of nuclei and disarrangement of hepatic cells in Figure 4 in contrast to the cord-like arrangement and pronounced sinusoid spaces for the liver section in Figure 7. X 800.

Fig. 8-11. Photomicrographs of hen livers that were freeze-mounted and stained with hematoxylin and eosin. The livers were analyzed and shown to contain 47.0, 28.0, 13.5 and 2.3% lipid, respectively (wet basis). Note the leakage of lipid to extracellular spaces in Figures 8 and 9. X 800.

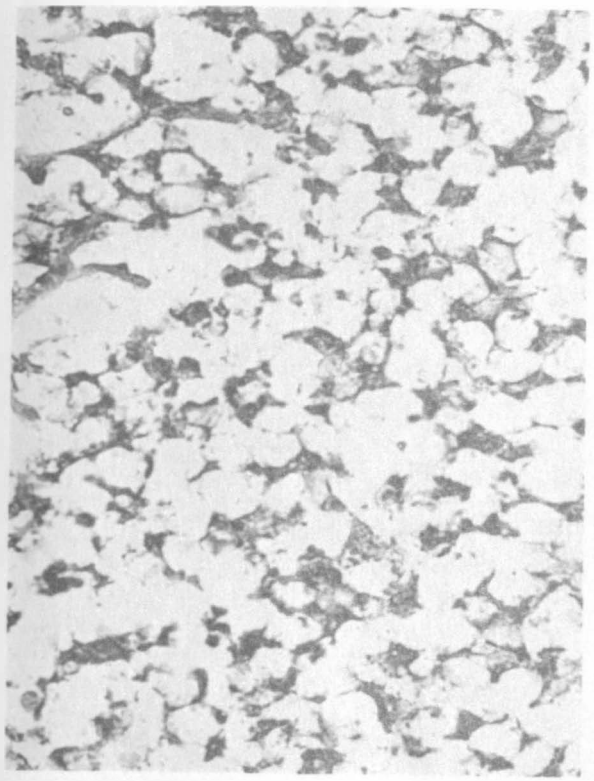


FIG. 8

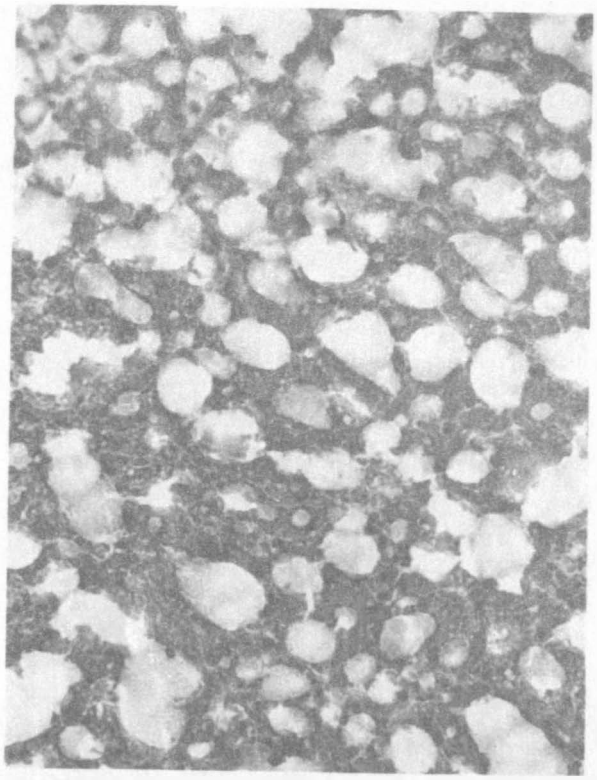


FIG. 9

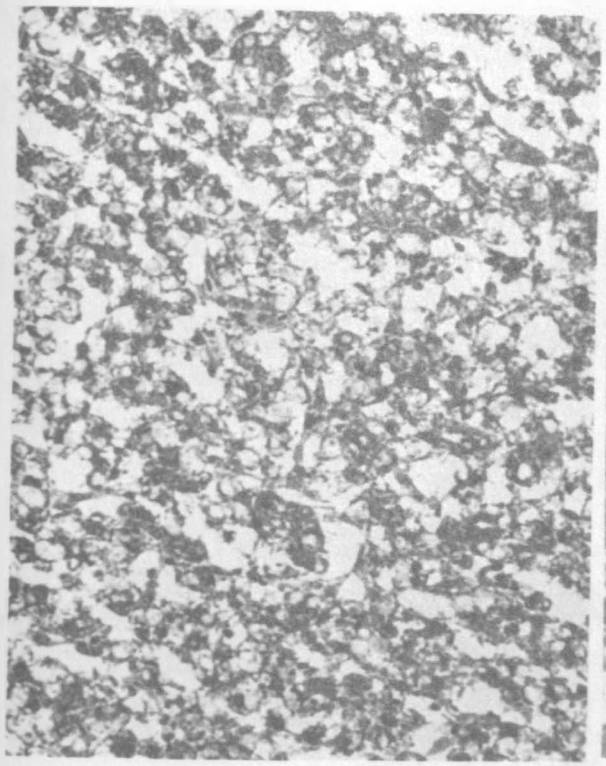


FIG. 10

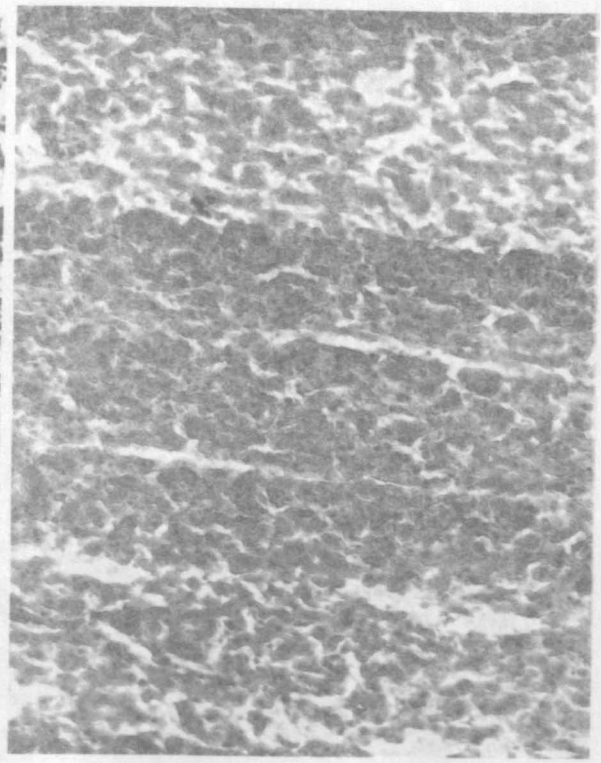


FIG. 11

Fig. 12-15. Photomicrographs of hen livers that were freeze-mounted and stained with oil red O. The livers were analyzed and shown to contain 47.0, 28.0, 13.5 and 2.3% lipid, respectively (wet basis). Note the leakage of lipid to extracellular spaces in Figures 12 and 13. Figure 15 shows the lack of liver lipid in a hen that was out of production for 17 days on an oats diet. X 800.

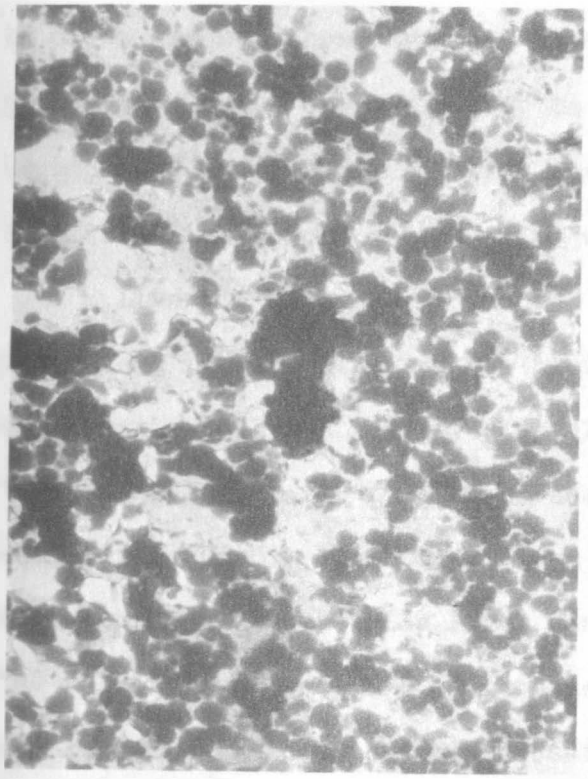


FIG. 12

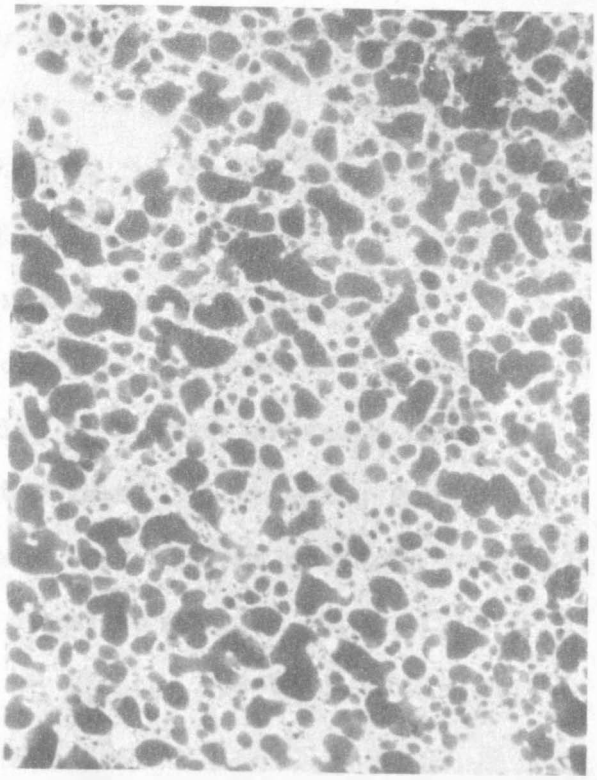


FIG. 13

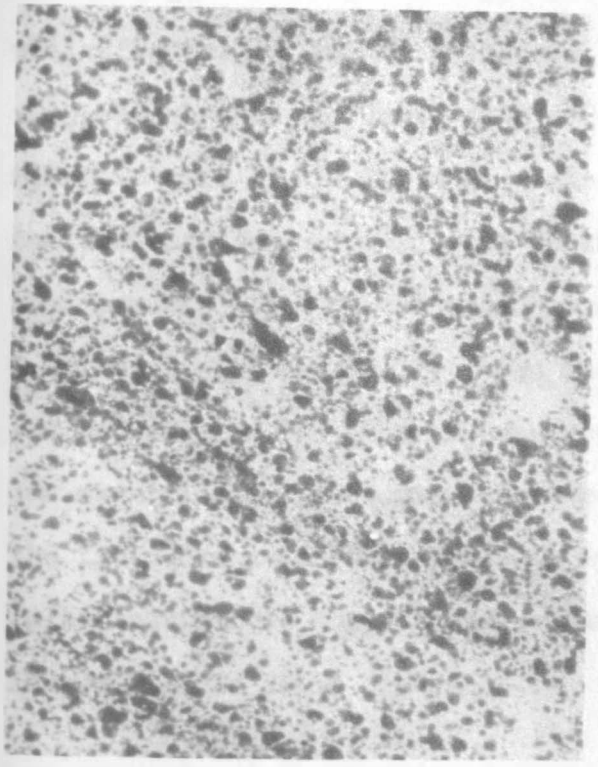


FIG. 14

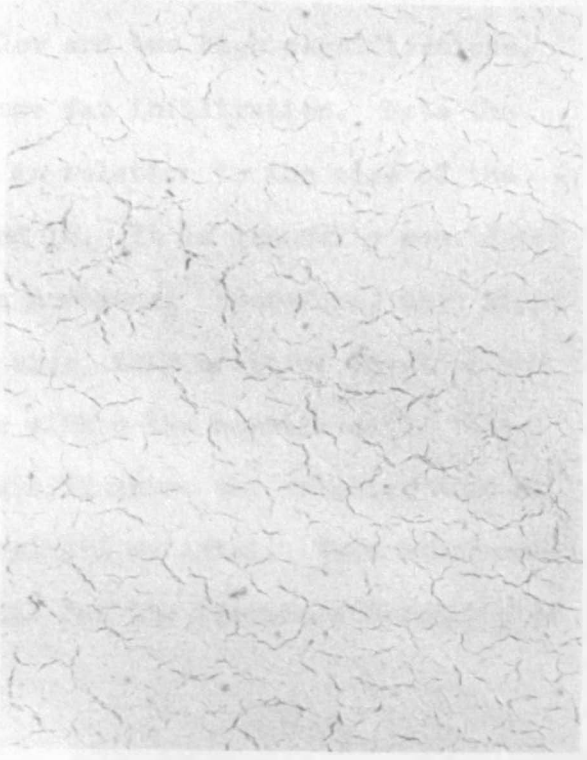


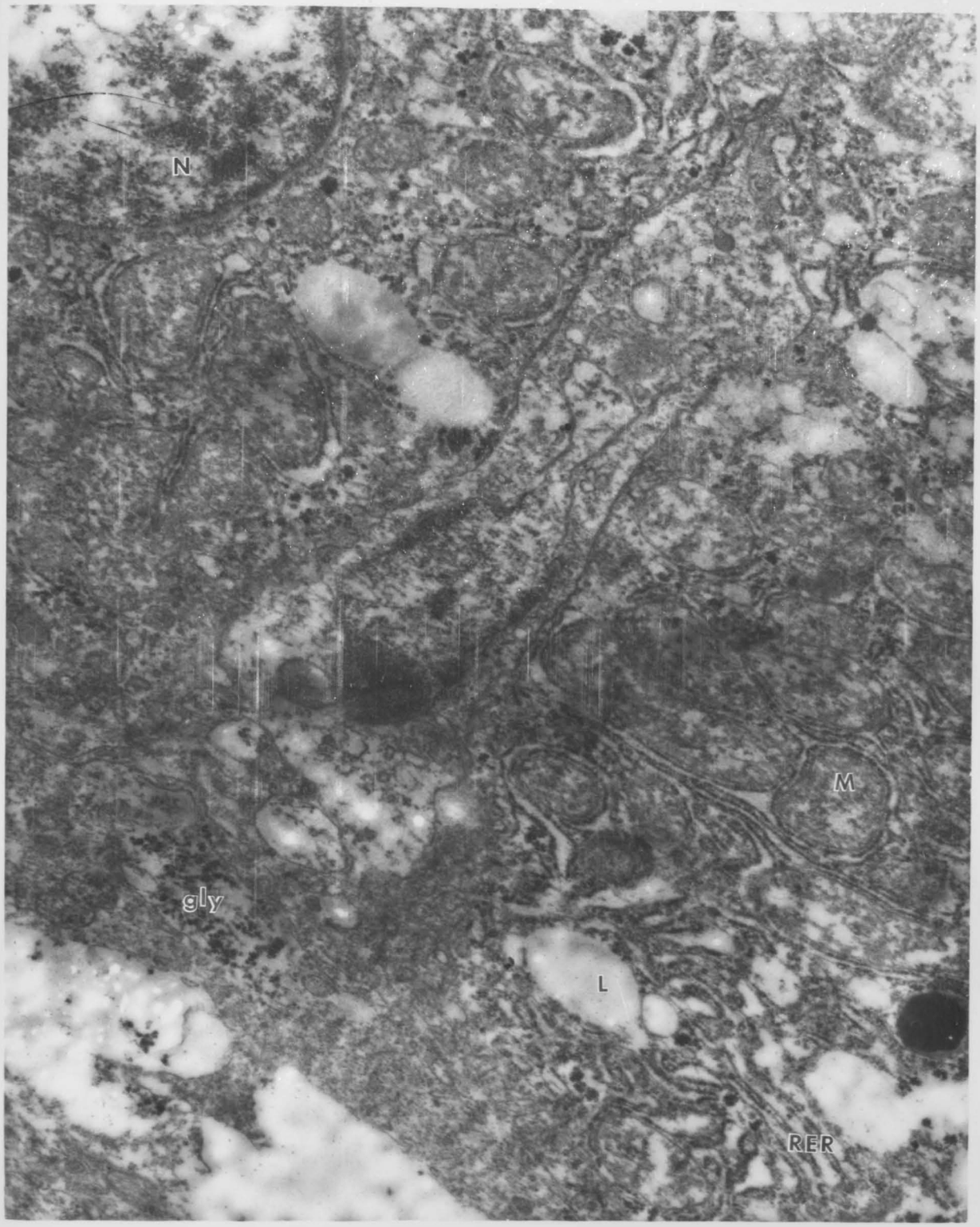
FIG. 15

17 days. As evidenced by these liver sections, fat infiltration *per se* should not be considered the diagnostic factor, since "normal" laying hens have relatively high levels of fat in the liver. However, excessive liver lipid accumulation will lead to FLHS.

Livers from two hens in good reproductive condition in Experiment 11 were selected for histological observation by electron microscopic analysis. A liver weighing 28 g and considered to be "normal" was compared to a liver that weighed 141 g coming from a hen with severe FLHS. The normal liver was analyzed and shown to contain 8.6% lipid, while the FLHS liver was found to contain 40.2% lipid. The normal liver was removed from an *ad libitum* fed bird, while the abnormal one was produced by force-feeding.

Figure 16 shows an electron micrograph of the normal liver, while Figures 17 through 19 show a low and two high magnifications, respectively, of the liver with severe fat infiltration. Note the relative size of the lipid droplets in relation to the size of the nucleus when comparing Figures 16 and 18. It is generally considered that hepatic lipid is not bound by a membrane. Therefore, when lipid droplets become abnormally large in size, they coalesce together and put pressure on the other organelles within the hepatic cell. The low magnification micrograph in Figure 17 shows the relative size of the lipid droplets in relation to nonlipid material. This unbalanced relationship more than likely accounts for the increased fragility of livers high in lipid content.

Fig. 16. Electron micrograph of a "normal" hen liver analyzed and shown to contain 8.6% lipid. Note the relative size of the lipid droplets to the size of the nucleus. Also note the general abundance of glycogen and endoplasmic reticulum. X 23900.


N
M
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L
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Fig. 17. A low magnification electron micrograph of a hen liver inflicted with FLHS that contained 40.2% lipid. Note the relative size of the lipid droplets in contrast to the amount of structural components present. The wrinkled condition of lipid areas result from its replacement with embedding material. This material does not section easily, resulting in a "chatter" condition. X 7170.

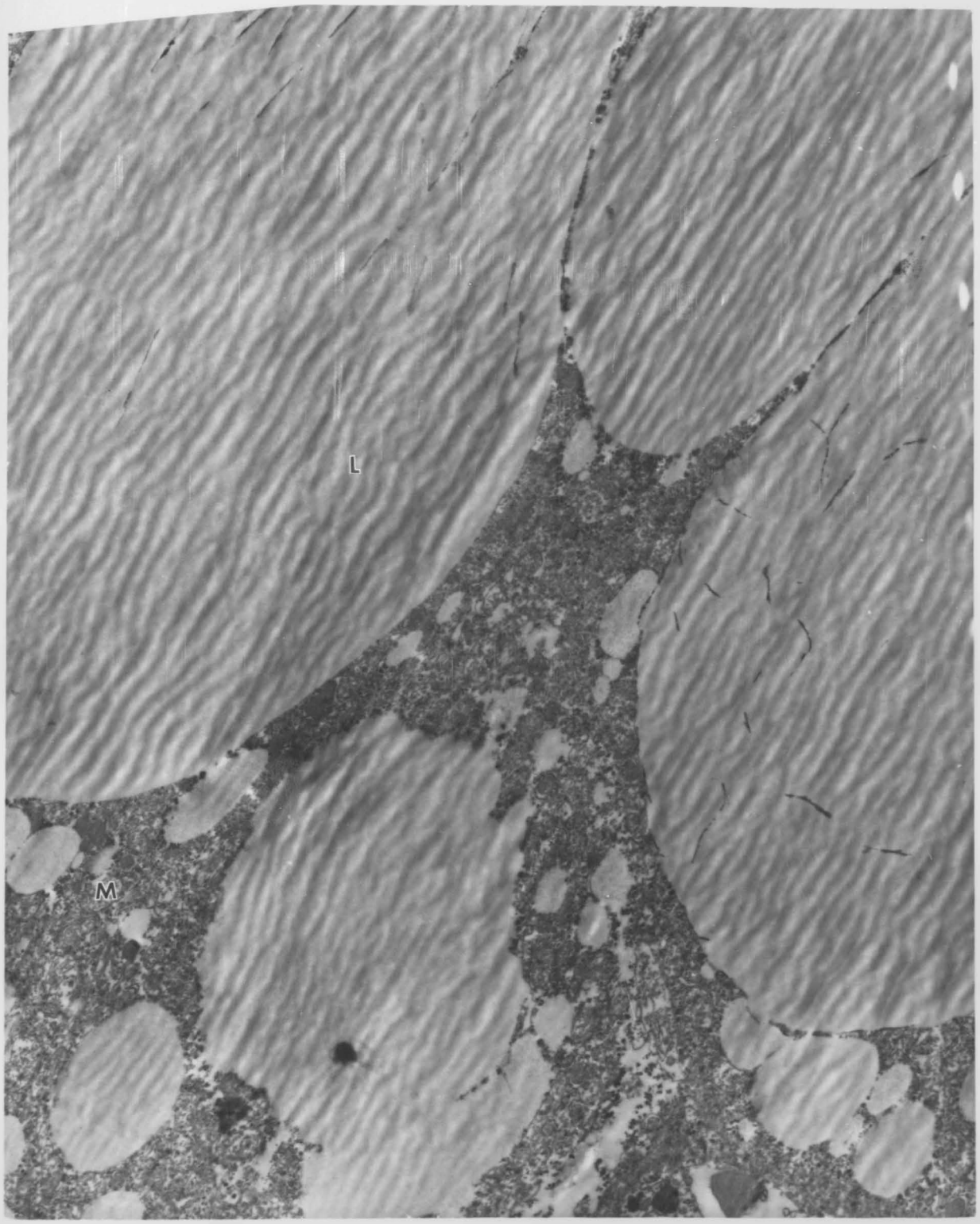
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Fig. 18. An electron micrograph of a hen liver inflicted with FLHS that contained 40.2% lipid. Crowding by the lipid droplets often caused the nucleus to be isolated from other organelles in the hepaticyte. X 23900.

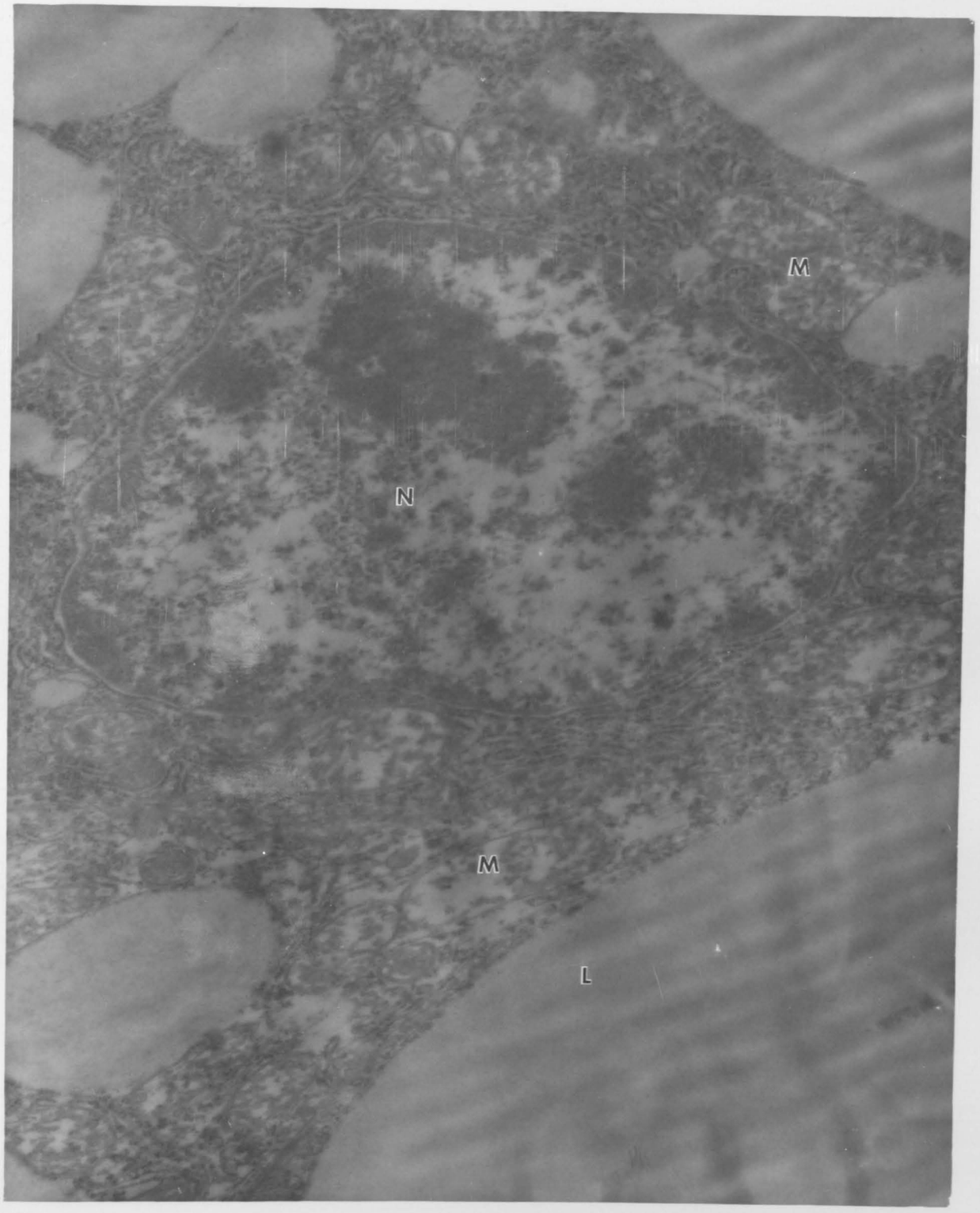
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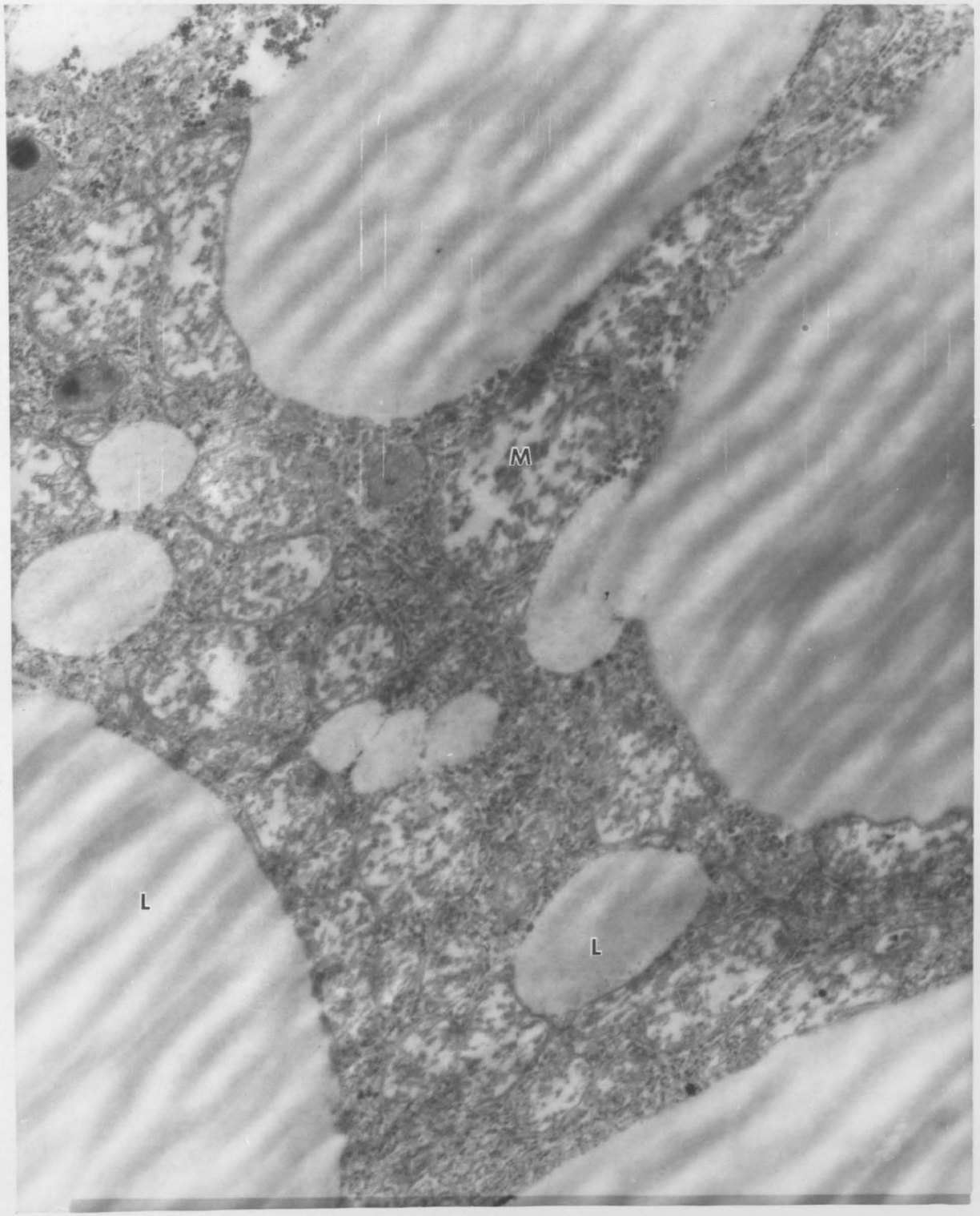
Fig. 19. An electron micrograph of a hen liver inflicted with FLHS that contained 40.2% lipid. The mitochondria appear swollen and lack the normal density of cristai in the matrix. X 23900.


M
L
L

Other hepaticyte organelles apparently change in appearance in hens with FLHS. Note the difference in the structure of the mitochondria between the two livers. The mitochondria in the liver from the treated hen appear swollen and have a relatively low density of cristae projecting into the matrix in comparison to the normal liver.

Another striking characteristic of the FLHS inflicted liver is its lack of rough endoplasmic reticulum. This lack of reticulum could account for the inability of the liver to maintain structural stability through protein synthesis in a hen inflicted with FLHS.

One of the functions of the liver is its ability to store large reserves of energy in the form of glycogen. The micrograph of the normal liver in Figure 16 shows numerous dense aggregates or "rosettes" of glycogen in many areas of the hepaticyte. These aggregates were generally found to be lacking in the liver of the FLHS inflicted hen.

GENERAL DISCUSSION

In each of the eleven experiments conducted in this study, one to three of five strains of birds were utilized in a cage production system to test several dietary factors for their effects on the incidence of FLHS. The dietary factors studied were choline, biotin and methionine supplementation; protein and fat level; fiber and grain source and the level of energy consumption. A force-feeding system similar to the one described by Wolford and Polin (1972b) was used in Experiments 5 through 11. It successfully produced the type of hemorrhages and steatosis within the hens' livers comparable to the condition "fatty liver syndrome" which was first described by Couch (1956) and more recently termed Fatty Liver-Hemorrhagic Syndrome (FLHS) by Wolford and Polin (1972a).

Choline supplementation of 1500 mg/kg of diet was tested in Experiments 1, 5, 9 and 10. The results generally confirm previous reports by Welch and Couch (1955), Soloma et al. (1965), Holmes and Kramer (1965) and Griffith et al. (1969) that excessive choline supplementation will, at times, increase egg production. Egg production increases of 1 to 11% were observed in these experiments with lesser effects generally observed in the shorter term force-feeding experiments. Reports in the literature indicate that the effectiveness of choline supplementation in preventing lipid accumulation in the liver is quite controversial. The results of choline supplementation on liver lipids are conflicting in this study, also. In Experiments 1 and 10, a

decrease of 40 to 50% total liver lipid was observed with the choline supplemented corn diets, while no effects were observed in Experiments 5 and 9. No effect was observed from choline supplementation of oats diets. Jensen et al. (1974b), Crawford et al. (1969) and Griffith et al. (1969) showed a significant effect of choline on decreasing liver fat, while other researchers (Bossard and Combs, 1970; Nesheim et al., 1969; Chah et al., 1975) were unable to show, with practical diets, any effect of choline on liver fat.

Biotin has been shown to eliminate mortality due to Fatty Liver-Kidney Syndrome in broiler chicks (Blair and Whitehead, 1974; Payne et al., 1974). Jensen et al. (1976b) found no benefit from biotin in reducing hepatic liver lipid in the laying hen. The results of this study generally agree with those of the latter reports. A significant decrease of 3.5 g of total liver lipid ($P < .05$) was observed in Experiment 1 with biotin supplementation which was due primarily to a decrease in liver size. However, no effect was observed for the biotin supplemented hens in Experiment 5.

Supplementing excess methionine and/or choline in Experiment 9 did not decrease liver lipids. In fact, methionine supplementation alone resulted in a significant ($P < .05$) increase in total liver lipids by an average of 11 g per liver. This increase was due primarily to a larger liver size. On the other hand, Roberson et al. (1970) and Griffith et al. (1969) found that supplementing methionine at levels higher than that required for egg production prevented the development of FLHS by lowering liver and abdominal fat.

The level of protein in the layer diet has been shown to have little effect on liver fat accumulation (McDaniel et al., 1959). Protein levels of 16 and 12% were used in Experiment 3 to test for their effect on production and liver lipids. Although the 16% diet supported a significantly higher rate of egg production, no differences were observed in any liver parameters measured due to protein level.

In Experiments 1 and 5, a high level of dietary fat (10% animal fat) was used to try to increase the incidence of FLHS. Weiss and Fisher (1957) had shown that levels of 5 to 10% animal fat fed to birds on litter resulted in several changes in lipid metabolism, including the production of friable and fatty livers. This high level of fat did not produce livers considered to be abnormally high in lipid in either Experiment 1 or 5. Force-feeding Experiments 7 and 8 tested the effects of 2, 5 and 8% added dietary fat. In both experiments, increases in fat level produced no significant increases in liver lipids, but rather there was a tendency for liver lipid to decrease as the dietary lipid level was increased.

Three sources of grain (corn, oats and wheat) were used in these studies to test for effects on FLHS. Experiment 2 compared all three sources in an ad libitum feeding regime. Experiment 6 compared corn and wheat in a force-feeding regime, while Experiment 10 compared corn and oats in a force-feeding regime. Conflicting results occurred between Experiments 2 and 6. In Experiment 2, the wheat-fed birds did not have significantly different liver lipid levels from the corn-fed birds. However, in Experiment 6, significantly ($P < .01$) less liver

lipid (6.4 g per liver) was observed for hens fed wheat as compared to hens fed corn. The diets were not fed isocalorically in either experiment and this may have accounted for some of the differences. Reports in the literature are contradictory for the effects from feeding wheat. Jensen et al. (1976e) found wheat diets to decrease total liver lipid by almost 50% of that of hens fed corn. However, Kim et al. (1976) found no significant differences in liver parameters measured after 20 weeks of feeding soft white wheat, hard red wheat or corn type diets.

Oats as the primary carbohydrate source resulted in significantly lower egg production in Experiments 2 and 10. Production averaged 10% less than for hens on the corn diet for the 13 production periods in Experiment 2. Evidence suggests that oats contain a factor necessary for normal lipid metabolism in laying hens, since no hemorrhages were noted in any oats-fed hens and total liver lipid was reduced by 50 to 75% as compared to that of corn-fed birds.

Experiments 4 and 11 involved the use of three fiber sources (cellulose, oat hulls and wheat bran) added at quantities to produce experimental diets of 4.7% total fiber. The control diet was calculated to contain 2.8% fiber, while all four diets were made isocaloric by the addition of fat at the necessary level. Feeding these isocaloric diets ad libitum or by the force-feeding method resulted in no significant differences in egg production or liver parameters. The results in these two experiments indicate that the bran or hull part of wheat and oats, respectively, is not the factor responsible for the liver lipid lowering effect of wheat and oats diets.

Undoubtedly, one factor in the gross accumulation of body fat and concurrent development of fatty livers is excessive caloric intake and/or its decreased utilization in cage type housing systems. This dietary factor caused the most dramatic effect on liver lipid levels in this study. Feed intake ranged from 114 to 162% of the control ad libitum birds for the seven force-feeding experiments in this study. This resulted in body weight gains of 90 to 350 g during the 3-week force-feeding periods. The average of total liver lipid ranged from 4.5 to 8.8 g per bird for the ad libitum fed birds (except hens on oats diets) in this study, while similar values for force-fed birds ranged from 12.3 to 35.2 grams. Increased levels of force-feeding (feed intake for force-fed birds ranged from 102 to 154 g per day for the seven experiments) did not necessarily result in higher hepatic lipid values. Other factors such as strain, age of hen or environmental temperature apparently accounted for some of the differences between experiments. In general, the hybrid strains had lower hepatic values than the Regional Control straightbred hens, and older hens had higher lipid values than younger hens. In addition, the highest lipid values were obtained in experiments terminated in April or May.

An additional factor evaluated in this study was the effect of strain on liver lipids. Experiments 1, 2, 3 and 5 evaluated the effects of three hybrid strains of birds, while Experiment 4 compared the Shaver hybrid strain and the Regional Control straightbred SCWL hen. In the four experiments tested, the Hyline strain accumulated less liver lipids than the other two strains used, with this decrease being highly

significant in Experiments 1 and 3. In Experiment 3, less mortality was observed for the Hyline strain due to FLHS. Although the straight-bred hens were not compared to all of the hybrid strains in this study, examination of individual data indicates a greater tendency for FLHS in the Regional Control hen.

Observations at the time of necropsy and later histological observations generally showed in this study that caged hens in normal egg production have a relatively high level of liver lipid, causing the liver to appear yellow or yellow-maghogany in color. When fat levels become excessively high, hemorrhages are more likely to occur. Histological examination verified these observations, with FLHS inflicted hens showing an abundance of intracellular and extracellular lipid in the liver, while hens out of production had livers almost devoid of lipid. Electron micrographs also verified this extreme abundance of lipid in livers of FLHS inflicted hens. The excessive lipid appeared to displace the nuclei, mitochondria and other cell organelles, which must have caused a disruption in normal liver functions. The liver appeared to have difficulty maintaining adequate glycogen storage in comparison to a liver with relatively less lipid content.

SUMMARY

In this study, eleven experiments were conducted to investigate the relationship of several dietary factors and bird strain on the incidence of Fatty Liver-Hemorrhagic Syndrome in caged laying hens. Four of the experiments used an ad libitum feeding regime, while the other seven used a force-feeding technique to increase the incidence of the disease.

The conclusions were:

1. Choline supplementation in excess of normal recommendations generally increases egg production in low protein (14%) diets. In two of four experiments, this supplementation in corn type diets resulted in a decrease of total liver lipids by 40 to 50%. No effect was observed in the other two experiments including choline supplementation of oats type diets.

2. Supplementing biotin has little beneficial effect in preventing FLHS.

3. The level of protein in the diet affected egg production but had no effect on the liver parameters tested.

4. Supplementing excessive methionine (.1%) to increase the availability of methyl groups did not decrease liver lipids but, in fact, resulted in a significant increase ($P < .05$) in liver lipids of 11 g per liver.

5. The level of added dietary animal fat (2 to 10%) did not increase liver lipid accumulation. A tendency for decreased liver lipid was noted instead.

6. Grain source significantly affected the incidence and degree of FLHS. Wheat-fed birds had significantly less liver lipid than hens on a corn diet in one experiment, while no effect was observed in a second experiment. Oats as the primary carbohydrate source prevented FLHS at feed intake levels as high as 150% of *ad libitum* feeding. Evidence suggests that oats contains a factor necessary for normal lipid metabolism in caged laying hens.

7. Evidence from two experiments indicates that the bran or hull part of wheat or oats, respectively, is not the factor responsible for the lowering effect of wheat and oats diets on liver lipid.

8. Excessive caloric intake caused the most dramatic effect on liver lipid levels in this study. Force-feeding increased total liver lipids and the incidence of hemorrhage by three- to fivefold. However, the response varied between experiments, indicating environmental factors play an important role in the incidence of FLHS.

9. Variation in genetic susceptibility to FLHS was noted in several experiments. The hybrid strains (Babcock B-300, Shaver 288, Hyline W-36 and DeKalb 231) used in this study had lower hepatic values than the Regional Control SCWL hens, with the Hyline strain generally showing more resistance to liver lipid accumulation.

10. Histological observations showed that hens in normal egg production generally have relatively high levels of liver lipid, indicating that liver lipid level *per se* was not the only factor that causes FLHS. However, photomicrographs and electron micrographs showed that excessive liver lipid accumulation will lead to FLHS.

Photomicrographs showed livers with excessive lipid to have intracellular as well as extracellular lipid present. Electron micrographs showed that excessive liver fat caused severe disarrangement of the hepatic nuclei and mitochondria, with glycogen storage being largely replaced by lipid storage.

REFERENCES

Abbott, O. D., and C. U. DeMasters, 1940. Choline in the diet of chickens. J. Nutr. 19:47-55.

Balloun, S. L., 1956. Choline and tallow in breeder hen diets. Poultry Sci. 35:737-738.

Barton, T. L., 1967. Fatty liver studies in laying hens. Ph.D. Thesis. Michigan State University, East Lansing, Michigan.

Barton, T. L., C. J. Flegal and P. J. Schaible, 1966. "Fatty Liver" syndrome in laying hens as influenced by protein energy ratios. Poultry Sci. 45:1068.

Blair, R., and C. C. Whitehead, 1974. An assessment of the factors associated with fatty liver and kidney syndrome in broilers. Proc. 15th World's Poultry Cong., New Orleans, Louisiana. pp. 380-382.

Bossard, E. H., and G. F. Combs, 1970. Lipotropic agents in broiler breeder rations. Poultry Sci. 49:599-601.

Bragg, D. B., J. Sim and G. C. Hodgson, 1973. Influence of dietary energy source on performance and fatty liver syndrome in White Leghorn laying hens. Poultry Sci. 52:736-740.

Chah, C. C., R. A. Nelson and C. W. Carlson, 1975. Fatty liver-hemorrhagic syndrome as affected by fat, choline and biotin supplementation. Poultry Sci. 54:1743.

Couch, J. R., 1956. Fatty livers in laying hens--a condition which may occur as a result of increased strain. Feedstuffs, 28(47):46.

Crawford, J. S., M. Griffith, R. A. Teekell and A. B. Watts, 1967. Choline requirement as it influences egg production and feed consumption in laying hens. Poultry Sci. 46:1249.

Crawford, J. S., M. Griffith, R. A. Teekell and A. B. Watts, 1969. Choline requirement and synthesis in laying hens. Poultry Sci. 48:620-626.

Dunnett, C. W., 1955. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Statist. Ass. 50:1096-1121.

Duke, M. J., R. K. Ringer and J. H. Wolford, 1968. Failure of plasma protein level to indicate developing fatty liver in chickens. Poultry Sci. 47:1098-1100.

Gish, C. L., F. A. Kummerow and L. F. Payne, 1949. Choline and ethanolamine requirements in the laying ration. Poultry Sci. 28:305-306.

Garlich, J. D., J. D. Olsen, W. E. Huff and P. B. Hamilton, 1975. Liver lipid content of twenty varieties of laying hens from three confinement systems. Poultry Sci. 54:806-813.

Griffith, M., A. H. Olinde, R. Schexnailder, R. F. Davenport and W. F. McKnight, 1969. Effect of choline, methionine and vitamin B_{12} on liver fat, egg production and egg weight in hens. Poultry Sci. 48:2160-2172.

Griffith, M., and R. Schexnailder, 1972. Evaluation of choline and some other nutrients on the liver fat of the laying hen. Poultry Sci. 51:1813.

Hafez, E. S. E., 1955. Differential growth of organs and edible meat in the domestic fowl. Poultry Sci. 34:745-753.

Hamilton, P. B., and J. D. Garlich, 1971. Aflatoxin as a possible cause of fatty liver syndrome in laying hens. Poultry Sci. 50:800-804.

Hamilton, P. B., and J. D. Garlich, 1972. Failure of vitamin supplementation to alter the fatty liver syndrome caused by aflatoxin. Poultry Sci. 51:688-692.

Harms, R. H., D. A. Roland, Sr. and C. F. Simpson, 1977. Experimentally induced "Fatty Liver Syndrome" conditions in laying hens. Poultry Sci. 56:517-520.

Harms, R. H., C. F. Simpson and B. L. Damron, 1972. Some new observations on "fatty liver syndrome" in laying hens. Avian Dis. 16:1042-1046.

Hartfiel, W., E. Greuel and H. Grassmann, 1970a. Weitere Ergebnisse zum Fettlebersyndrom bei Legehennen. Arch. fur Geflugelk. 5:190-193.

Hartfiel, W., E. Greuel and R. M. Wegner, 1970b. Untersuchungen zum Fettlebersyndrom von Boden-und Kafighaltung. Arch. fur Geflugelk. 4:140-143.

Holmes, C. E., and C. Y. Kramer, 1965. Vitamin B_{12}, choline and methionine in laying diets. Poultry Sci. 44:1381.

Humason, G. L., 1972. Animal Tissue Techniques. W. E. Freeman and Co., San Francisco, California.

Ivy, C. A., and M. C. Nesheim, 1973. Factors influencing the liver fat content of laying hens. Poultry Sci. 52:281-291.

Jensen, L. S., J. M. Casey, S. I. Savage and W. M. Britton, 1976a. An association of hardness of water with incidence of fatty liver syndrome in laying hens. Poultry Sci. 55:719-724.

Jensen, L. S., C. H. Chang and D. V. Maurice, 1976b. Effect of biotin and niacin on lipid content of livers in the laying hen. Poultry Sci. 55:1771-1773.

Jensen, L. S., C. H. Chang and D. V. Maurice, 1976c. Improvement in interior egg quality and reduction in liver fat in hens fed brewer's dried grains. Poultry Sci. 55:1841-1847.

Jensen, L. S., C. H. Chang and D. V. Maurice, 1976d. Liver lipid accumulation and performance of hens as affected by cage density and initial body weight. Poultry Sci. 55:1926-1932.

Jensen, L. S., C. H. Chang and R. D. Wyatt, 1976e. Influence of carbohydrate source on liver fat accumulation in hens. Poultry Sci. 55:700-709.

Jensen, L. S., L. Falen and C. H. Chang, 1974a. Effect of distillers dried grains with solubles on reproduction and liver fat accumulation in laying hens. Poultry Sci. 53:586-592.

Jensen, L. S., D. V. Maurice and C. H. Chang, 1977. Relationship of mineral content of drinking water to liver lipid accumulation in laying hens. Poultry Sci. 56:260-266.

Jensen, L. S., G. W. Schumaier, A. D. Funk, T. C. Smith and L. Falen, 1974b. Effect of selenium and lipotropic factors on liver fat accumulation in laying hens. Poultry Sci. 53:296-302.

Johnson, E. L., 1954. Vitamin B_{12} requirements of hens as affected by choline and penicillin. Poultry Sci. 33:100-107.

Kim, L. M., M. B. Patel, S. J. Reddy and J. McGinnis, 1976. Effect of different cereal grains in diets for hens on production parameters and liver fat content. Poultry Sci. 55:520-530.

Leveille, G. A., and D. J. Bray, 1970. The lack of effect of dietary inositol in depressing liver lipids in the hen. Poultry Sci. 49:327-329.

Lucas, H. L., L. C. Norris and G. F. Heuser, 1946. Observations on the choline requirements of hens. Poultry Sci. 25:373-375.

Marthedal, H. E., G. Velling and B. Tylling, 1974. Fatty liver and kidney disease in chickens in Denmark. Proc. and Abstr. 15th World's Poultry Cong. p. 382.

McDaniel, A. H., J. H. Quisenberry, B. L. Reid and J. R. Couch, 1959. The effect of dietary fat, caloric intake and protein level on caged layers. Poultry Sci. 38:213-219.

N.R.C., 1971. Nutrient Requirements of Poultry. 6th ed. (revised). Natl. Acad. Sci., Washington, D. C. pp. 15-23.

Nesheim, M. C., C. A. Ivy and M. J. Norvell, 1969. Some observations on fatty livers in laying hens. Cornell Nutr. Conf. pp. 36-41.

Nesheim, M. C., M. J. Norvell, E. Ceballos and R. M. Leach, Jr., 1971. The effect of choline supplementation of diets for growing pullets and laying hens. Poultry Sci. 50:820-831.

Owings, W. J., S. L. Balloun, W. W. Marion and J. M. J. Ning, 1967. The influence of dietary protein level and bird density in cages on egg production and liver fatty acids. Poultry Sci. 46:1303.

Payne, C. G., P. Gilchrist, J. A. Pearson and L. A. Hemsley, 1974. Involvement of biotin in the fatty liver and kidney syndrome of broilers. Brit. Poultry Sci. 15:489-498.

Pearce, J., 1972. The lack of effect of dietary inositol supplementation on egg production and liver lipid metabolism in the laying hen. Poultry Sci. 51:1998-2001.

Polin, D., and J. H. Wolford, 1973. Spontaneous vs. induced fatty liver-hemorrhagic syndrome in adult female chickens. Feedstuffs, 45(21):40-42.

Polin, D., and J. H. Wolford, 1976. Various types of diets, sources of energy and positive energy balance in the induction of fatty liver-hemorrhagic syndrome. Poultry Sci. 55:325-334.

Price, J. D., A. H. McDaniel, D. N. Smith, Jr., J. H. Quisenberry, B. L. Reid and J. R. Couch, 1957. The effect of energy and protein levels on egg production, feed efficiency and some lipid constituents of blood and liver of caged layers. Poultry Sci. 36:1316-1321.

Quisenberry, J. H., L. A. Young and P. U. L. Murphy, 1967. The fatty liver syndrome in commercial layers. Poultry Sci. 46:1308-1309.

Ragland, W. W., D. W. Cardin, D. S. Snetsinger, R. A. Zimmerman and D. E. Greene, 1970. Effect of inositol on lay performance and body characteristics. Poultry Sci. 49:1430.

Reedy, L. M., 1968. Some clinical observations on the fatty liver syndrome (FLS) in laying hens. Proc. Texas Nutr. Conf. 23:80.

Ringrose, R. C., and H. A. Davis, 1946. Choline in the nutrition of laying hens. Poultry Sci. 25:646-647.

Roberson, R. H., V. Trujillo and D. W. Francis, 1970. The effect of methionine, thiouracil, dienestrol acetate and thyroprotein on the development and prevention of fatty livers in pullets. Poultry Sci. 49:1431.

Roland, D. A., Sr., and H. M. Edwards, Jr., 1971. Effect of essential fatty acid deficiency and type of dietary fat supplementation on biotin-deficient chicks. J. Nutr. 101:811-818.

Schexnailder, R., and M. Griffith, 1973. Liver fat and egg production of laying hens as influenced by choline and other nutrients. Poultry Sci. 52:1188-1194.

Shivaprasad, H. L., and R. G. Jaap, 1977. Egg and yolk production as influenced by liver weight, liver lipid and plasma lipid in three strains of small bodied chickens. Poultry Sci. 56:1384-1390.

Skinner, J. L., J. H. Quisenberry and J. R. Couch, 1951. High efficiency and APF concentrates in the ration of the laying fowl. Poultry Sci. 30:319-324.

Soloma, A. E., C. R. Creger and J. R. Couch, 1965. Effect of feeding varying levels of fat and choline chloride to laying hens. Poultry Sci. 25:646-647.

Steel, R. G. D., and J. H. Torrie, 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, New York.

Weiss, H. S., and H. Fisher, 1957. Plasma lipid and organ changes associated with the feeding of animal fat to laying chickens. J. Nutr. 61:267-280.

Welch, B. E., and J. R. Couch, 1955. Homocystine, vitamin B_{12}, choline and methionine in the nutrition of the laying fowl. Poultry Sci. 34:217-222

Wills, J. R., and J. E. Savage, 1974. Ultrastructure and lipid composition of livers from hens with hemorrhagic livers. Poultry Sci. 53:1992.

Wolford, J. H., 1971. The effect of temperature and iodinated casein on liver lipids of laying chickens. Poultry Sci. 50:1331-1335.

Wolford, J. H., and D. Murphy, 1972. Effect of diet on fatty liver-hemorrhagic syndrome incidence in laying chickens. Poultry Sci. 51:2087-2094.

Wolford, J. H., and D. Polin, 1972a. Laboratory duplication of fatty liver-hemorrhagic syndrome: A photographic description. Feedstuffs, 44(32):41-42.

Wolford, J. H., and D. Polin, 1972b. Force-feeding: A technique for nutritional physiological research. Poultry Sci. 51:1888.

Wolford, J. H., and D. Polin, 1972c. Lipid accumulation and hemorrhage in livers of laying chickens. A study on fatty liver-hemorrhagic syndrome (FLHS). Poultry Sci. 51:1707-1713.

Wolford, J. H., and D. Polin, 1974. Induced fatty liver-hemorrhagic syndrome and accumulation of hepatic lipid in force-fed laying chickens. Poultry Sci. 53:65-74.

Wolford, J. H., and D. Polin, 1975. Effect of inositol, lecithin, vitamins (B_{12} with choline) and iodinated casein on induced fatty liver-hemorrhagic syndrome in laying chickens. Poultry Sci. 54:981-991.

Wolford, J. H., R. K. Ringer, C. C. Sheppard, T. L. Barton and C. J. Flegal, 1971. Fatty liver syndrome. A photographic description. Feedstuffs, 43(4):28-29.